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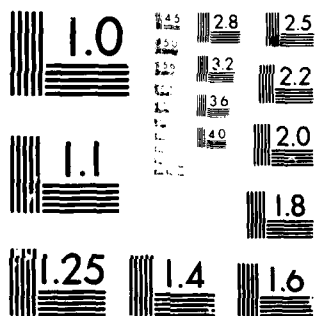
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DA Project 3M161102BS01 - Research of Military Injuries and Diseases

PROPHYLACTIC METHODS IN THE PREVENTION OF DISEASES AMONG ARMY PERSONNEL

ANNUAL PROGRESS REPORT

DECEMBER 1979

by

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Presidio of San Francisco, CA., 94129

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## ABSTRACT

Surveillances to determine the etiologic agents of Acute Respiratory Disease (ARD) in Basic Combat Trainees (BCTs') were accomplished during Fiscal Year 1979. Included in these studies were 4,536 trainees hospitalized with ARD at 10 BCT Forts (including the Navy Training Center - San Diego) and a geographical variety of non-BCT military installations. Virus isolations and serological studies indicated 12.6% of ARD hospitalizations were caused by Adenoviruses (Adenovirus 21 = 60.4%, Adenovirus 7 = 5.3%, Adenovirus 4 = 29.5%, Adenovirus 3 = 4.8% of total Adenovirus isolates), 3.2% were caused by Influenza (Flu A = 87.3%, Flu B = 12.7% of total Flu cases): Mycoplasma Cocksackie Virus, Polio and Herpes Virus collectively caused 1% of ARD hospitalizations, and 83.2% were caused by undetermined agents. From the 4,536 samples for isolations received, there were 467 Adenovirus isolations (252 = Adenovirus 21s', 22 = 7s', 123 = 4s', 20 = 3s'), 50 Influenzae (all Flu As' H<sub>3</sub>N<sub>2</sub> and H<sub>1</sub>N<sub>1</sub>), 107 Poliomyelitis, 12 Herpes Simplex Viruses, 1 Echo 9 and 1 Cocksackie A-9. From the 2,404 paired serum samples studied, there were 288 Adenovirus Seroconversions, 356 Flu As', 77 Flu Bs', and 36 Mycoplasmae. Adenovirus Vaccines 4 and 7 were administered between October 1978 and May 1979 at all Forts except Forts Leonard Wood and Mc Clellan where they were administered the year round. Laboratory titrations of virus in the vaccine pills indicated acceptable potency, 106.0 TCID<sub>50</sub> for Adenovirus 7 and 105.0 for Adenovirus 4 Vaccine. Adenovirus 21 Vaccine was not administered during the year. The Influenza Vaccine that was administered during the year was a trivalent product containing A/Texas/1/77 (H<sub>3</sub>N<sub>2</sub>), A/Russian/90/78 (H<sub>1</sub>N<sub>1</sub>) and B/Hong Kong/5/72, 20 mcg each; and monovalent A/Russian/90/78 60 mcg for recruits only.

Field studies to determine the immunogenicity of Adenovirus Vaccines were conducted utilizing paired sera collected from 398 individuals at 6 BCT Forts. Accumulative results indicated the type 7 Vaccine was 80.9% immunogenic and the type 4 was 69.6% immunogenic.

The microtiter serum neutralization test was evaluated for use in identifying type specific antibodies to Adenoviruses in paired sera from 201 patients from the Adenovirus Surveillance Program. This procedure would possibly replace the Complement-Fixation Test which only detects the Adenovirus group antigen and therefore does not distinguish between Adenovirus Serotypes. Heterotypic reactions were detected, which interfered with a correct determination of specific serotype totals. From 201 patients in this study, 77 Adenoviruses were isolated (Adenovirus 21 = 48, Adenovirus 4 = 17, Adenovirus 7 = 12), however, all 201 had 4-fold or greater CF rises. Seventy (70) of these 201 patients had > 4-fold rises to only one serotype (either Adenovirus 4, 7 or 21, 35%) while 109 patients (55%) had > 4-fold rises to two or all three Adenovirus Serotypes. Twenty (20) patients (10%) lacked > 4-fold rises in Adenovirus Neutralizing Antibodies to any of the three types.

An Adenovirus type 5 transformed Human Embryonic Kidney (HEK-T) cell line was compared with other cell lines that are currently used for the Adenovirus Surveillance Program. Of the 5 parallel cell lines used, Human Embryonic Kidney (HEK), Human Embryonic Kidney-Transformed (HEK-T), Rhesus Monkey Kidney (RMK), Vera Monkey Kidney (VMK), Human Amnion Normal FL (FL), and the Institute of Medical Research-90 cells (IMR-90), HEK cells were the most sensitive to Adenoviruses type 4, 7, 21, 2, 3, 19 and a variety of other viruses tested (Herpes, Polio 3, Influenza A, Coxsackie A9, A16, B2, B3, B4, Cytomegalovirus, Mumps, Echo 7, 9, 11).

Two major groups of individuals were surveyed for penicillinase-producing Neisseria gonorrhoeae (PPNG) during the year. One group consisted of military persons showing Urethritis symptoms while undergoing separation physicals, and had served a last tour of duty in the Philippines, Korea, Japan, Hawaii, or stations in the Pacific. Another group consisted of persons who had shown treatment failures on penicillin therapy for 3-7 days. Gonococcal (GC) isolates were tested for penicillin sensitivity, and resistant strains were farther assayed for beta-lactimase production. Chlamydial isolation procedures and antibody determinations were also performed. Of the 13 Gonococcal isolates from 55 patients, only 1 was resistant to penicillin and it did not produce beta-lactimase. All 39 specimens cultured for Chlamydia were negative.

Monthly Meningococcal Carrier Surveys were performed at Fort Ord during the year to monitor the prevalence of specific serological types. The average monthly carrier rate was 32.5%. A high of 49% in March and a low of 9% in June were encountered. Group B was the most prevalent serogroup (61% of isolates) during the year. There was one group B disease during the year; the patient's unit was treated with Minocycline Prophylaxis.

## FOREWORD

<sup>P</sup> This ~~progress~~ report describes the results of efforts by this study group during the past ~~fiscal year~~<sup>2</sup> to research methods of forecasting, detecting, and preventing upper respiratory and infectious diseases before they caused group illnesses in military populations, especially troop training units. Therefore, our greatest efforts have been directed towards those diseases that cause the largest loss of training days among recruits and advance trainees, and those that have the greatest potential of costing the government large amounts for hospitalizations or sick leaves. Such diseases are those caused by the Adenoviruses, Influenza, Mycoplasma, Coxsackieviruses, N. Meningitidis, and agents of Urethritis. A significant amount of effort has been directed towards prophylactic procedures. It is intended that the variety of approaches and techniques described herein will lead to further pathways even more effective in coping with these problems. <sup>41</sup>

We owe profound thanks to individuals in the LAMC, Virology and Bacteriology Reference Laboratories (LAIR), the Microbiology staffs at the other three Area Reference Laboratories at Fort Sam Houston, Fort Gordon and Fort Meade, and the Preventive Medicine Teams at Forts Ord, Dix, Knox, Wood, Sill, Benning, Jackson, Bliss, Gordon, Mc Clellan and the Navy Training Center, San Diego, without whose help portions of these investigative studies could not have been accomplished. Very special gratitude is owed to our Secretary Ms. Emma Devora, who endured the unenviable tasks of assisting in the preparation of tables, figures and graphs, and typing drafts leading to this final manuscript.

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## ACUTE RESPIRATORY DISEASE SURVEILLANCE

### INTRODUCTION

The etiologic agents of upper respiratory infections vary percent wise from year to year, but Acute Respiratory Disease (ARD) continues to be the principal cause of morbidity in military training units. These surveillance studies as conducted during the past ARD season, and for 16 years before at training Forts in CONUS have aided in signalling early warnings of pending epidemics and provided indicators for vaccine administration (1). These surveillance studies were performed on hospitalized basic combat trainees (BCT's) at 9 BCT Forts in CONUS, and on hospitalized and quartered patients at 1 Navy Training Center - San Diego, and 1 Advance Individual Training Fort (AIT) - Fort Ord.

### MATERIALS AND METHODS

The subjects for this surveillance were BCTs, AITs, and permanent party persons totaling 4,536 at military installations throughout CONUS. Nine of the installations were BCT Forts as indicated in Figure 1. Within 12 hours following hospitalization for ARD retro-uvula swabs and acute-phase sera were obtained from each individual. Convalescent-phase sera were collected 14 to 21 days later. Paired sera were obtainable on 2,404 individuals. A number of individuals completed BCT and transferred before convalescent serum collection dates. Some were lost because of short discharges and AWOLS. The swabs were placed in charcoal viral transport media and held at 50C until examined (usually 2-4 days) at the Medical Laboratory. Virus isolations were accomplished in tissue culture utilizing human embryonic (HEK) and rhesus monkey embryonic kidney (MEK) monolayers, and embryonated chicken eggs. Study sera were received as pairs after the convalescent collections, and they were used for detecting diagnostic rises in antibody titer by the complement fixation test to Adenovirus, influenza A and B, Coxsackievirus and Mycoplasma. Adenovirus sero-types were identified by microtiter neutralization tests utilizing Hela cell cultures. Influenza isolates were tested for by guinea pig erythrocyte hemadsorption, and sero-types were identified using specific anti-sera. Mycoplasma infections were identified by seroconversions; isolations and species determinations were accomplished only on those individuals who were also included in studies reported elsewhere in this report. Other viral serologies and isolation techniques were employed when indicated by isolated outbreaks, unusual isolates or for other diagnostic aids.

## ACUTE RESPIRATORY DISEASE SURVEILLANCE

### RESULTS AND DISCUSSION

Acute respiratory disease hospitalization rates per hundred per week during Fiscal Year 1979 at 10 Forts are indicated in Figure 1. Causative agents are discussed in a later paragraph. The highest rates occurred at Fort Leonard Wood where during the 2nd week of January it reached 5.8/100/week, and during the 3rd week of April 6.4/100/week. The April rates were among female trainees. ARD rates among female trainees at Fort Dix reached 4.1/100/week during the 3rd week of February; male rates stayed around 2/100/week. The ARD rates at Fort Sill spiked to 5.4/100/week during the 2nd week of January and remained low for the rest of the year. Fort Knox was the only other Fort that experienced significant ARD - 3.6/100/week in December and January. The other Forts remained below 3/100/week throughout the year. Adenovirus 4 and 7 oral vaccine pills containing  $10^5$  and  $10^6$  TCID<sub>50</sub> of Virus respectively, and Influenza Trivalent Vaccine containing A/Tex/1/77, A/Russ/90/78, and B/HK/5/72 20 mcg each, plus a Monovalent A/Russ/90/78 vaccine containing 60 mcg were administered during the dates indicated in Figure 1. Females did not receive Adenovirus vaccines.

Figure 2 indicates the ARD hospitalization rate per hundred per month, and etiologic agents at Fort Dix during Fiscal Year 1979. The same information is shown for Fiscal Year 1978 for comparison. The peak rate in February of approximately 1.7/100/week does not compare with this severely hard hit Fort in February of last year. Adenovirus 4 and 7 vaccines were administered from 1 September 1978 to 1 May 1979. Influenza vaccine was given 1 October 1978 to 3 April 1979. During the year 16.7% ARD hospitalizations were caused by adenovirus infections (53 Adenovirus 4s', 6 Adenovirus 7s', and 49 Adenovirus 21s' were isolated), 3.1% were caused by Influenza (56 Flu As' and 15 Bs'), 1.3% by Mycoplasma (9), Herpes (4), Polio (14) and Echo 9 (1), and 78.8% ARD etiology was not determined.

The male and female combined ARD hospitalization rates at Fort Jackson rose from approximately 1.0 to 2.8/100/week, which is a monthly peak of >1.7/100/month in February as indicated by Figure 3. As indicated by Figure 1 females contributed the greatest amount to these hospitalizations. A peak of 1.5/100/month last year occurred in February also as indicated in Figure 3. Adenovirus 4 and 7 vaccines were administered from 1 October 1978 to 1 April 1979, and influenza vaccine was given throughout the year. Adenoviruses (3 Adenovirus 21s', 3 Adenovirus 4s' and 1 Adenovirus 7 were isolated with 81 sero-conversions) caused 2.3% of ARD hospitalizations, 0.5% were caused by Influenza (56 Flu A's, and 15 B's), 0.5% (2) by Mycoplasma and Polio Virus, and 96.7% of the causes were not determined.

## ACUTE RESPIRATORY DISEASE SURVEILLANCE

As Figure 4 indicates, ARD hospitalization rates were low during the year for both males and females at Fort Mc Clellan. However, there were 22 Influenza A infections that caused an ARD rate of 1.0/100/month in February. Influenza caused 16.0% of ARD admissions. Only 1 Adenovirus 7 was isolated; 0.9% of admissions caused by Mycoplasma and Polio and 81.1% etiology was not determined. Adenovirus 4 and 7 vaccines were given to males only, and Influenza vaccine was given to male and females as indicated in Figure 4.

The greatest number of male ARD hospitalizations at Fort Gordon, 2.8/100/week, occurred in January. These were mostly females as indicated by Figure 1. As indicated by Figure 5, the greatest monthly average ARD hospitalization rate was 0.6/100/month in January. Figure 5 also indicates there were more ARD hospitalizations during Fiscal Year 1978. Adenovirus 4 and 7 vaccines were given 1 October to 1 April 1979, and Influenza vaccine was given the year round. During the year, 8.1% of ARD hospitalizations were caused by Adenoviruses (10 Adenovirus 21's, 3 Adenovirus 4's, and 11 Adenovirus 7's were isolated), 9.0% were caused by Influenza (86 Flu A's and 20 B's), 1.1% (5) by Mycoplasma, and 66.5% ARD etiology was not determined.

Figure 6 indicates ARD hospitalizations at Fort Knox during Fiscal Year 1979. A peak rate of 2.1/100/month was reached in January. The highest rate of >3/100/month occurred last year in February also. Adenovirus 4 and 7 vaccines were administered from 1 October to 1 May, and Influenza vaccines were given the year round as indicated in Figure 6. During the year 20.1% ARD hospitalizations were caused by Adenoviruses (107 Adenovirus 21's, 20 Adenovirus 3's and 42 Adenovirus 7's), 0.8% by Influenza (16 Flu A's, and 3 Flu B's), 0.7 by Mycoplasma, and 78.4% of etiology was not determined.

Fort Leonard Wood experienced the greatest number of ARD hospitalizations throughout the year of any of the BCT Forts. This ARD was caused by both the Adenovirus and Influenza viruses. A peak rate of 3.8/100/month occurred in January, however a significant amount of ARD occurred throughout February, March and April. Female training began in March at Fort Leonard Wood and the majority of ARD during March and April was contributed by female trainees; this is indicated by Figure 1. Adenovirus 4 and 7 vaccines which had been administered throughout the previous year continued throughout 1979. Adenoviruses (41 Adenovirus 21's, 16 Adenovirus 4's and 1 Adenovirus 7 were isolated) caused 8.5% of ARD hospitalizations, 2.4% were caused by Influenza (50 Flu A's and 15 Flu B's), 1.3% by other agents (12 Mycoplasma, 2 Herpes, 14 Polio), and 87.8% were not determined as indicated by Figure 7.

## ACUTE RESPIRATORY DISEASE SURVEILLANCE

Figure 8 indicates ARD hospitalization rates at Fort Sill. There were few admissions until the 2nd week in January when a significant number (5.2/100/week) of BCTs' became ill. The average monthly rate at that time was 2.5/100/month. Influenza A and B were identified by isolation and serology as the most probable cause of these hospitalizations. Adenovirus 4 and 7 and Influenza vaccines were given during dates indicated by Figure 8. As indicated by this figure Influenza infections caused the majority of ARD hospitalizations during the previous year. Adenoviruses (4 Adenovirus 21's and 2 Adenovirus 4's were isolated) caused 13.3% of the ARD, 13.3% was caused by Influenza (9 Flu A's and 4 Flu B's), and 73.3% of causes were not determined.

While Fort Bliss experienced the highest ARD hospitalization rate of any BCT Fort during a single week the previous year 1978 (25/100/week, or 8.85/100/month), the same severity was not experienced this past year. A peak rate of >1.5/100/month in March was caused by a variety of agents. Adenoviruses (1 Adenovirus 21, 1 Adenovirus 7, and 2 Adenovirus 4's were isolated) caused 12.5% ARD hospitalizations, 5.6% were caused by Influenza (5 Flu A's and 1 B), 6.9% were caused by other agents e.g., 1 Mycoplasma, 1 Coxsackie A-9 and 4 Polios, and 75% of the etiology could not be determined. Both Adenovirus and Influenza vaccines were administered between October 1978 and April 1979 as indicated by Figure 9.

Fort Benning began BCT during the last part of November, and a number of ARD hospitalizations in December raised the rate to >2/100/week. The monthly rate as indicated by Figure 10 was 1/100/month. The ARD rate remained below 2.0 for the balance of the year. Of the ARD hospitalizations that did occur, Adenoviruses (36 Adenovirus 21's and 2 Adenovirus 4's were isolated) caused 16.8%, Influenza (21 Flu A's and 2 Flu B's) caused 4.5%, other agents e.g., Mycoplasma and Polio caused 0.7%, and 78% etiology was not determined.

The ARD hospitalization rate at Fort Ord remained at 1/100/month or below throughout the year as indicated by Figure 11. Very few specimens were submitted for isolation, and none for serological studies. Therefore the etiology determination may not be accurate. The only isolations were 17 polio viruses, and these could have been vaccine viruses. Because of a lack of specimens submitted the undetermined etiology on ARD hospitalizations and quarters patients was 97.2%. Influenza vaccine was administered at Fort Ord between October and May.

Acute Respiratory Diseases hospitalized and quartered patients totaling 121 at the U. S. Navy Training Center, San Diego were included in these Surveillance Studies. Only 1 Adenovirus 7, 3 Poliomyelitis viruses, and 4 Herpes Simplex viruses were isolated. Paired sera for serologies were not received.

## SUMMARY

The over-all ARD picture of all of the BCT Forts that were surveyed during the year is summarized by Figure 12. The average ARD hospitalization rate per hundred per month for Fiscal Year 1979 is indicated. Fiscal year 1978 is shown for comparison. The highest average ARD hospitalization rate was approximately 1.6/100/month in January. Most of the Influenza admissions occurred January thru March, and Adenovirus admissions occurred for the most part January thru September. Usually Adenovirus and Influenza Vaccines were administered October thru April, however, some Forts vaccinated the year round. Including all of the Forts, an average of 12.6% of ARD was caused by the Adenoviruses, 3.2% by Influenzae, 1% by other agents e.g., Mycoplasmae, Herpes, Poliomyelitis, and Cocksackie viruses; and 83.2% were caused by agents that were not determined.

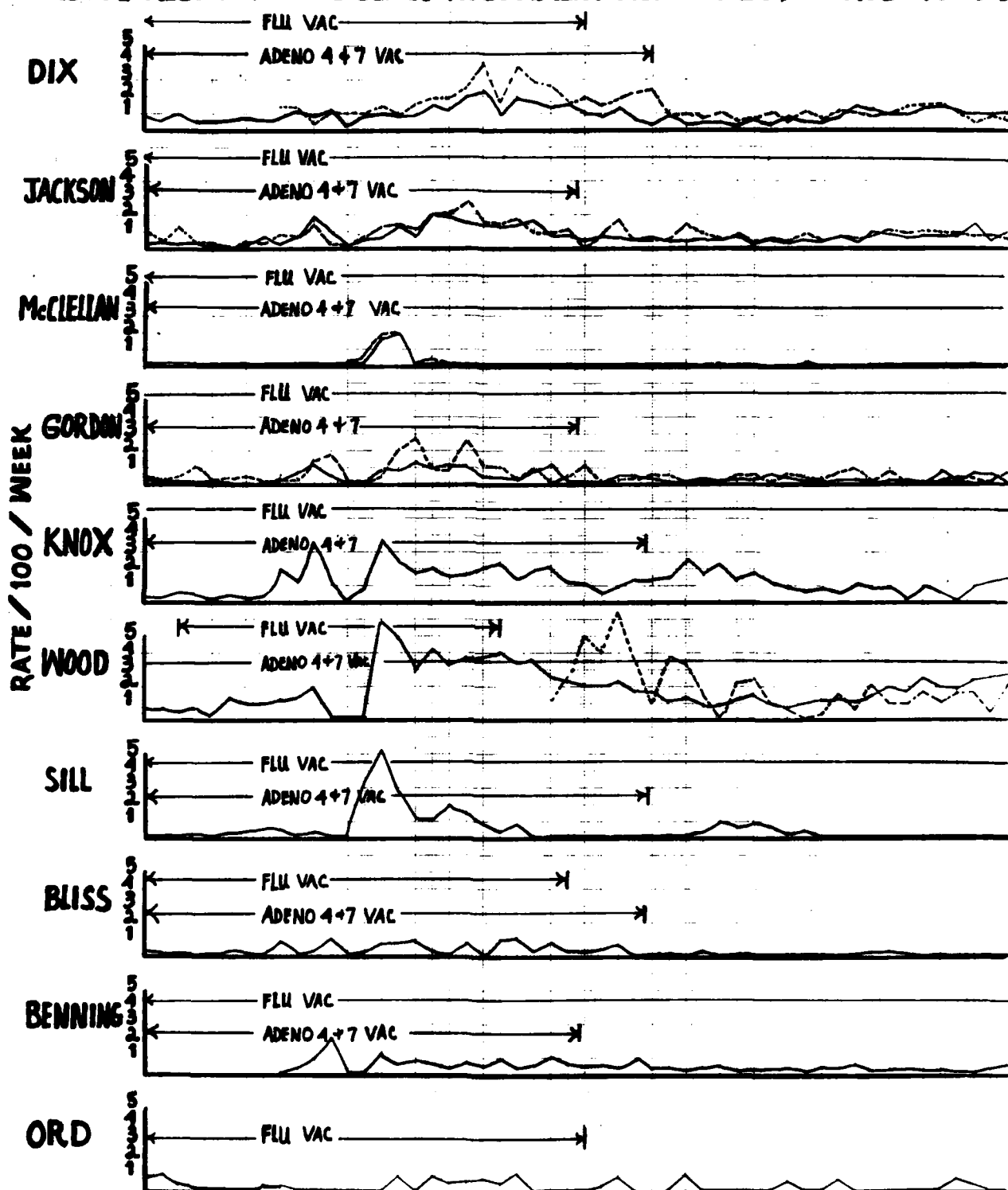
Table 1 summarizes causative agents by isolations and seroconversions obtained from the total samples taken from Acute Respiratory Disease patients at 10 training Forts, and one Naval Training Center that were surveyed, and 10 other military stations that experienced Upper Respiratory Disease problems. From 4,536 individuals, 588 virus isolations were made (13%). Of the isolations, 71% (417) were Adenoviruses (60.4% Adenovirus 21's, 5.3% Adenovirus 7's, 29.5% Adenovirus 4's, 4.8% Adenovirus 3's), 8.5% were Influenza (all A's), 18.1% Polio viruses, 2% Herpes, and 0.3% Echo and Cocksackie Viruses. Of the 2,404 serum pairs studied, 757 (31%) seroconversions were obtained. Adenoviruses comprised 288 (38%) of these seroconversions, 356 (47%) were due to Influenza A, 77 (10%) were to Influenza B, and 36 (4.7%) to Mycoplasma. The Influenza A agents were predominantly H<sub>1</sub>N<sub>1</sub> A/Brazil, however H<sub>3</sub>N<sub>2</sub> A/Texas was also represented. It could not be determined if the 107 Polio viruses were vaccine viruses.

## REFERENCE

1. Smith, C.D., Col., et al. Prophylactic Methods in the Prevention of Diseases Among Army Personnel. Annual Progress Reports 1970-1979.

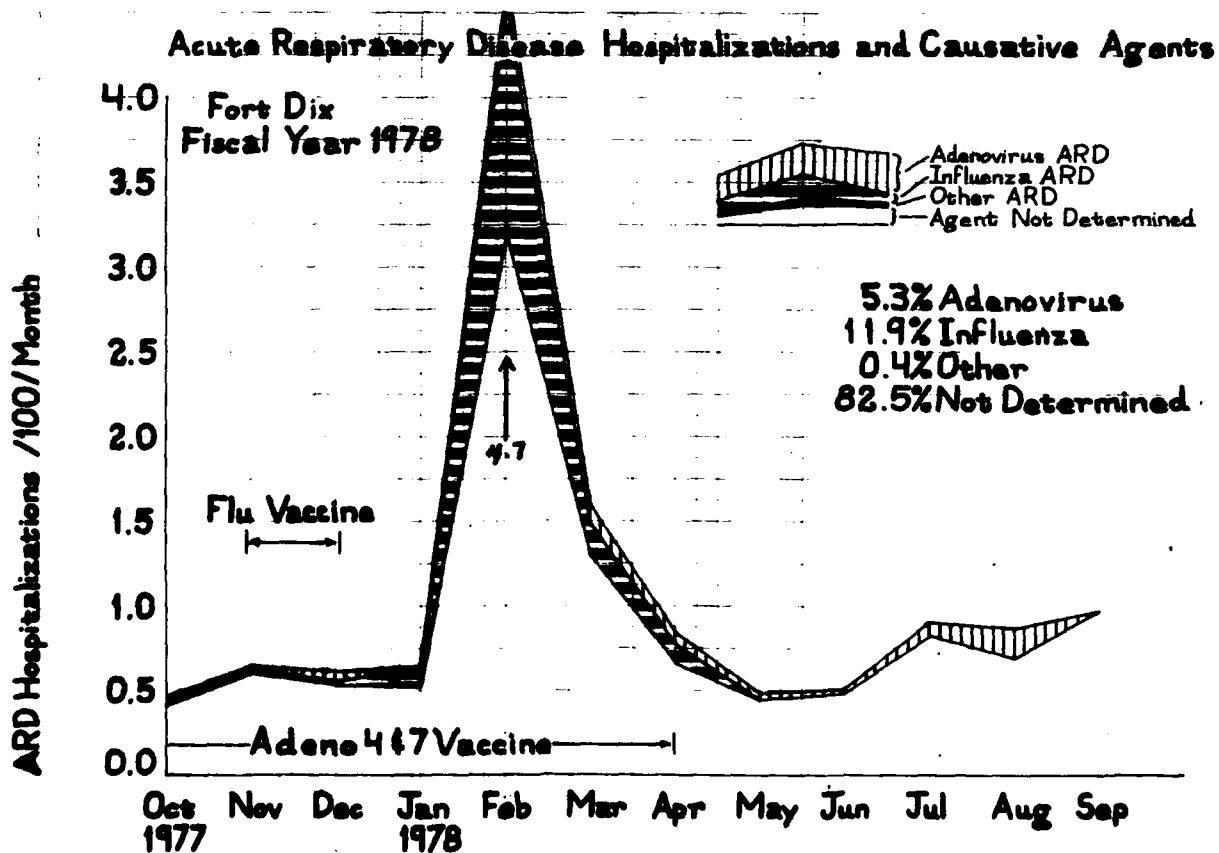
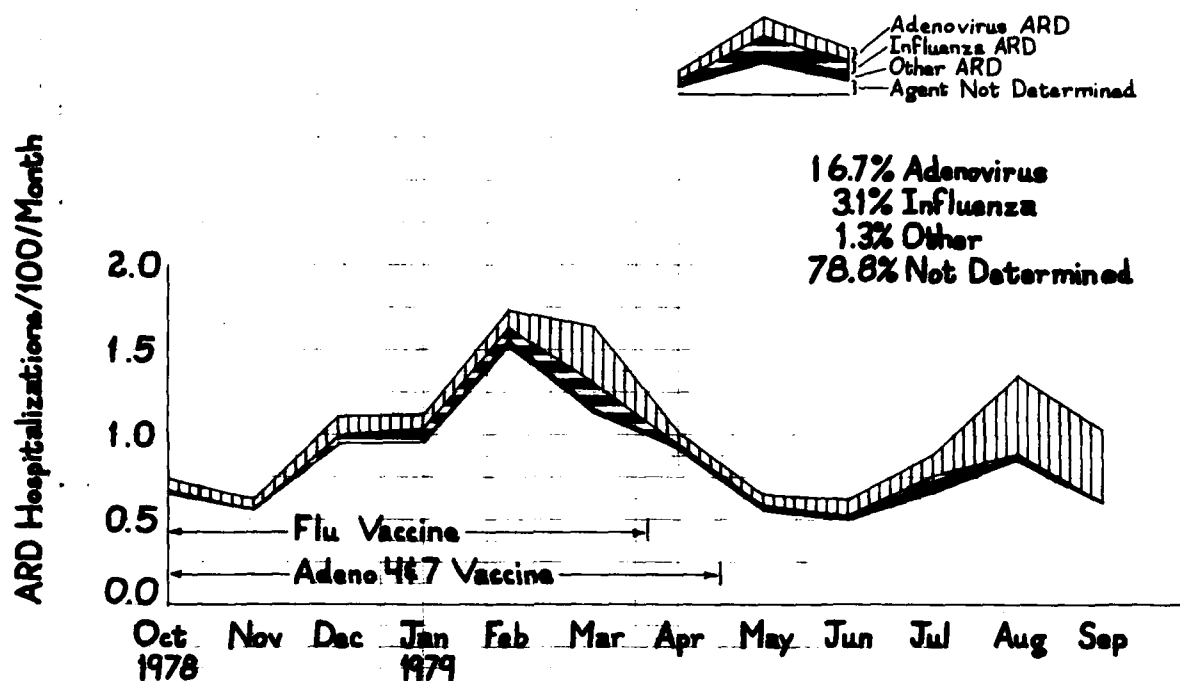
FIGURE 1

# ACUTE RESPIRATORY DISEASE HOSPITALIZATIONS AT BCT FORTS-FY '79

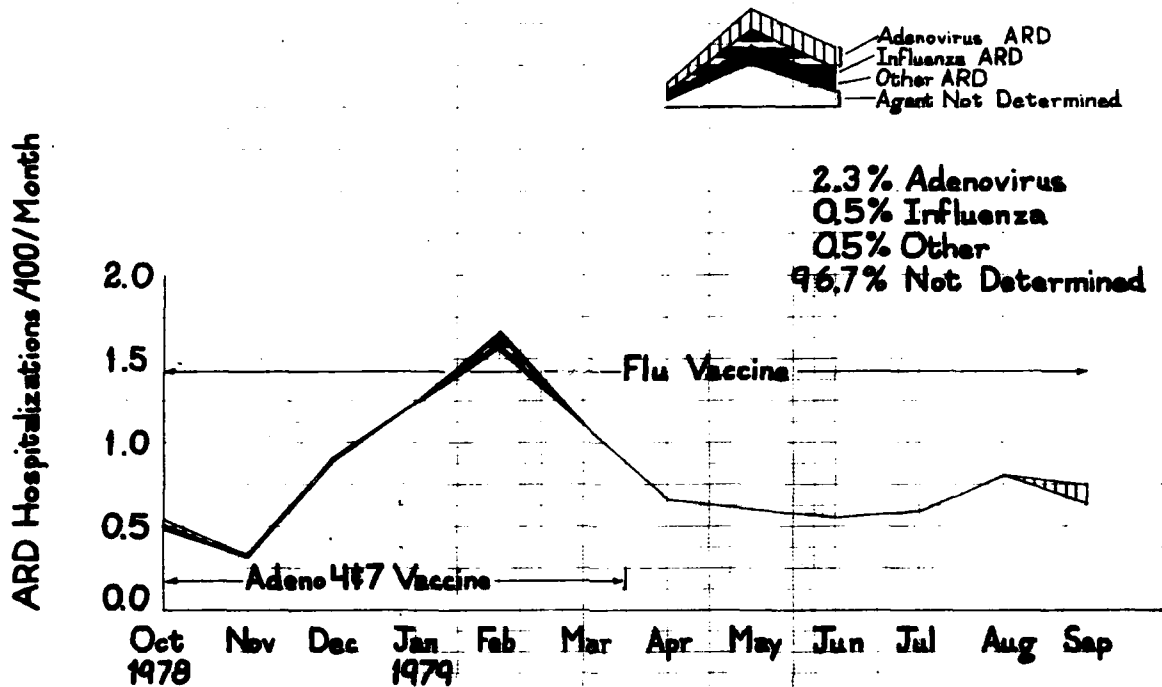


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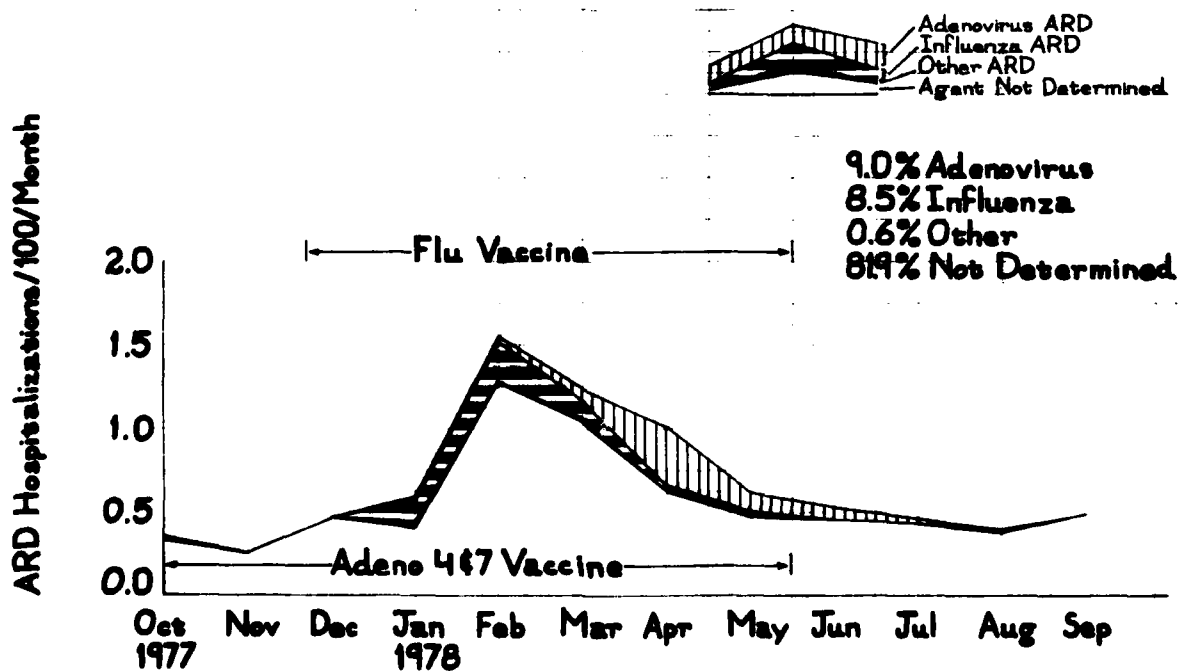
**FIGURE 2**  
**Acute Respiratory Disease Hospitalizations and Causative Agents**  
**Fort Dix—Fiscal Year 1979**



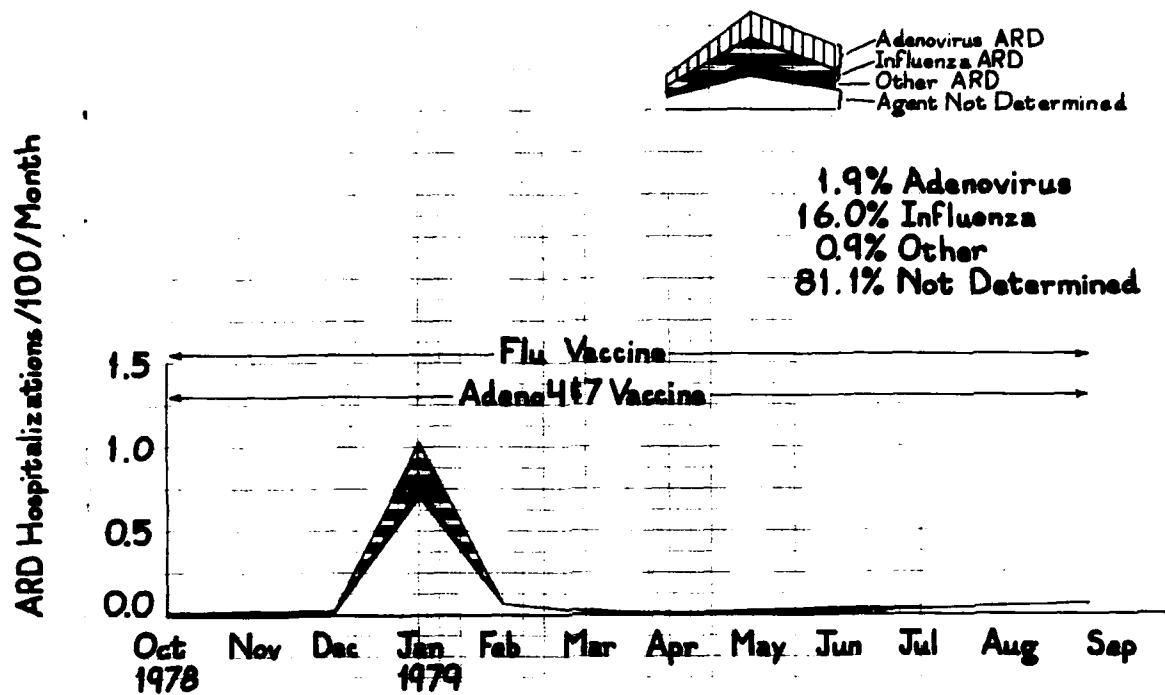
**FIGURE 3**  
**Acute Respiratory Disease Hospitalizations and Causative Agents**  
**Fort Jackson - Fiscal Year 1979**



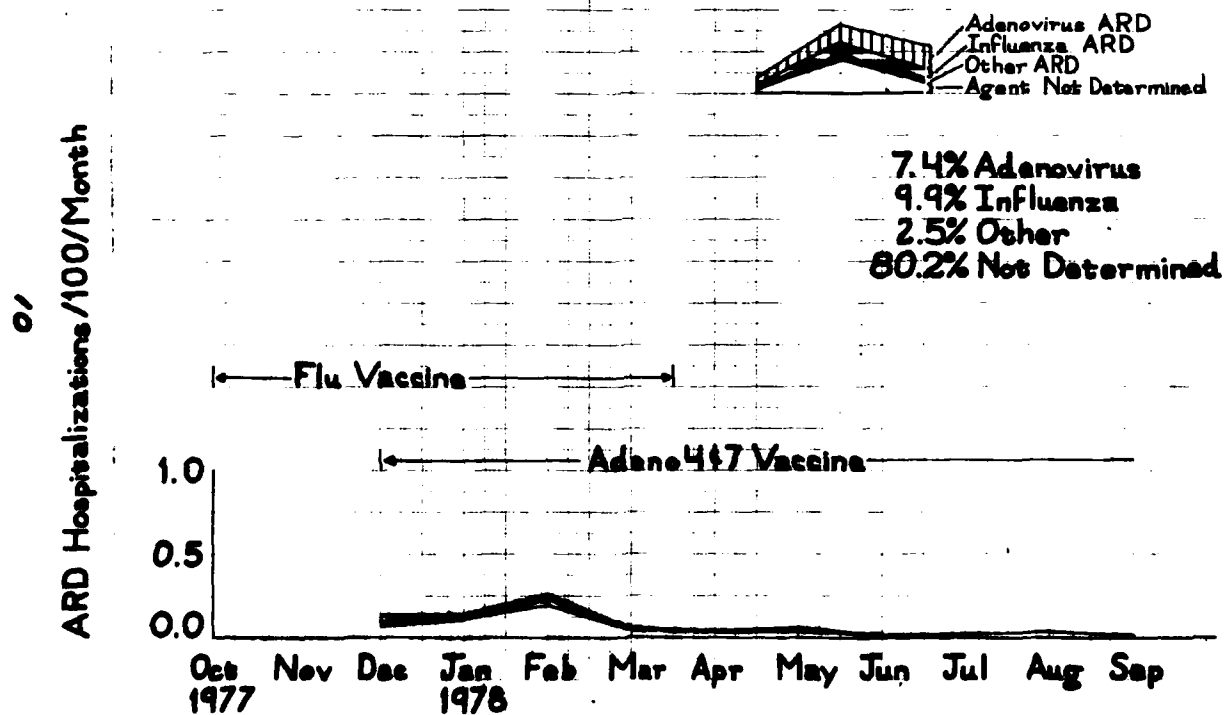
**Acute Respiratory Disease Hospitalizations and Causative Agents**  
**Fort Jackson - Fiscal Year 1978**



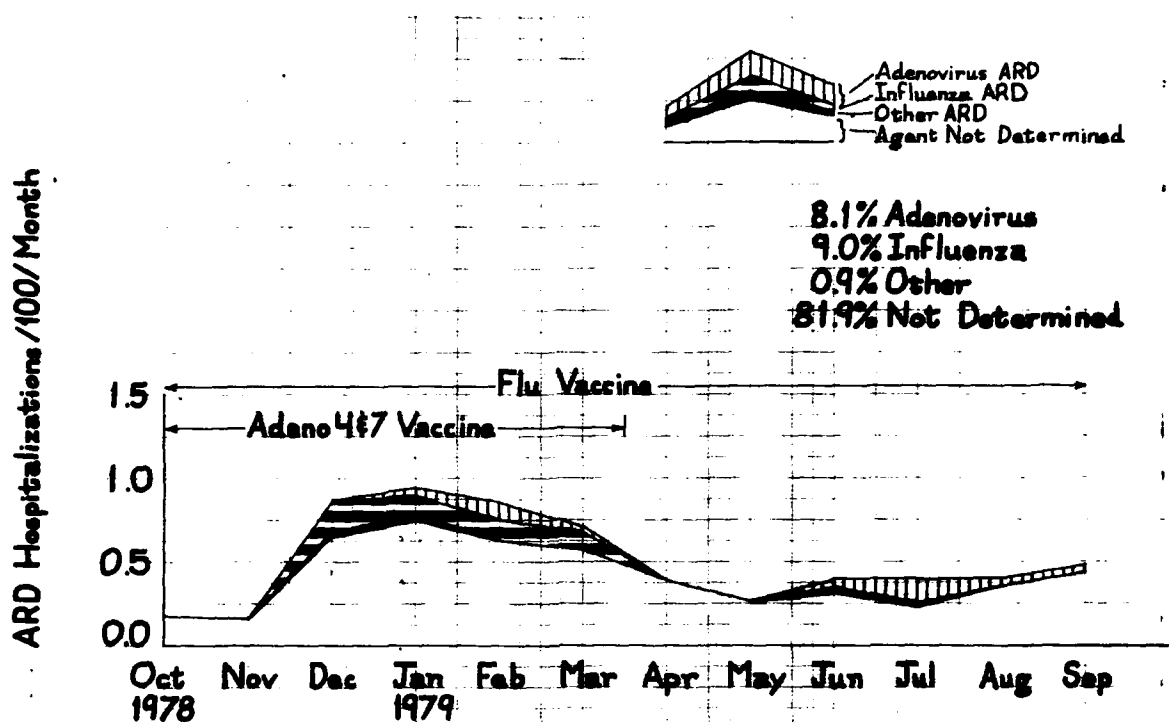
**FIGURE 4**  
**Acute Respiratory Disease Hospitalizations and Causative Agents**  
**Fort McClellan—Fiscal Year 1979**



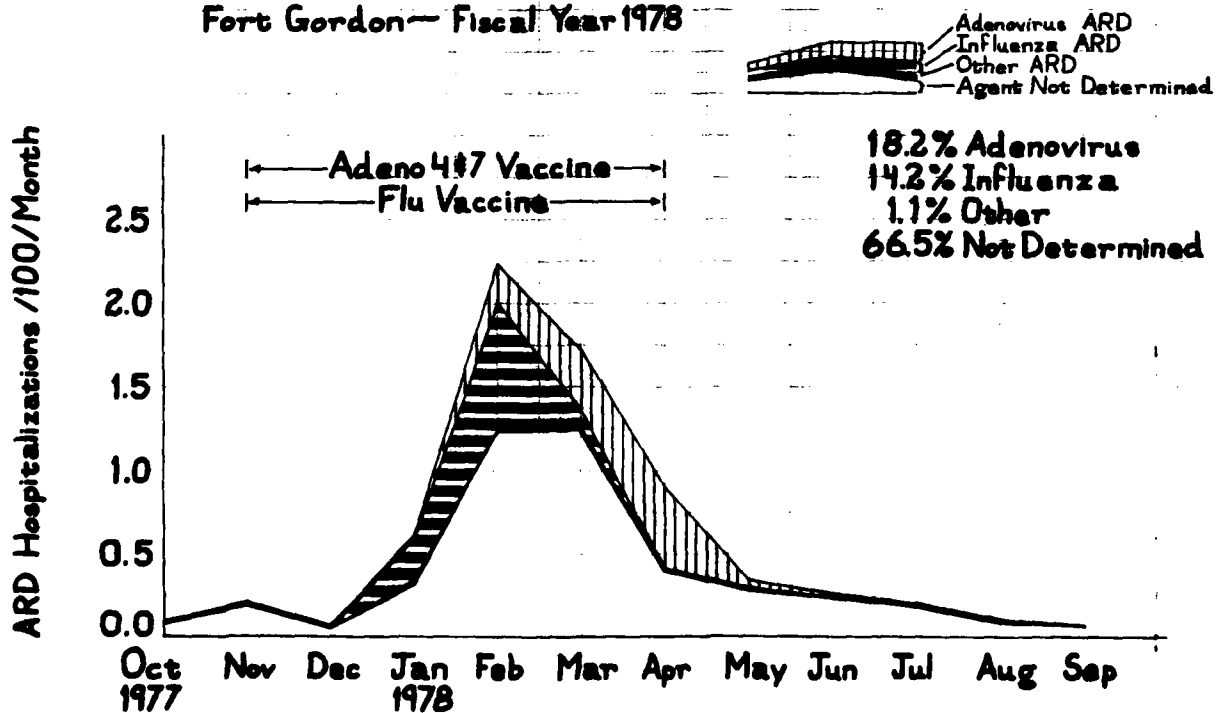
**Acute Respiratory Disease Hospitalizations and Causative Agents**  
**Fort McClellan—Fiscal Year 1978**



**FIGURE 5**  
**Acute Respiratory Disease Hospitalizations and Causative Agents**  
**Fort Gordon — Fiscal Year 1979**



**Acute Respiratory Disease Hospitalizations and Causative Agents**  
**Fort Gordon — Fiscal Year 1978**



**FIGURE 6**  
**Acute Respiratory Disease Hospitalizations and Causative Agents**  
**Fort Knox—Fiscal Year 1979**

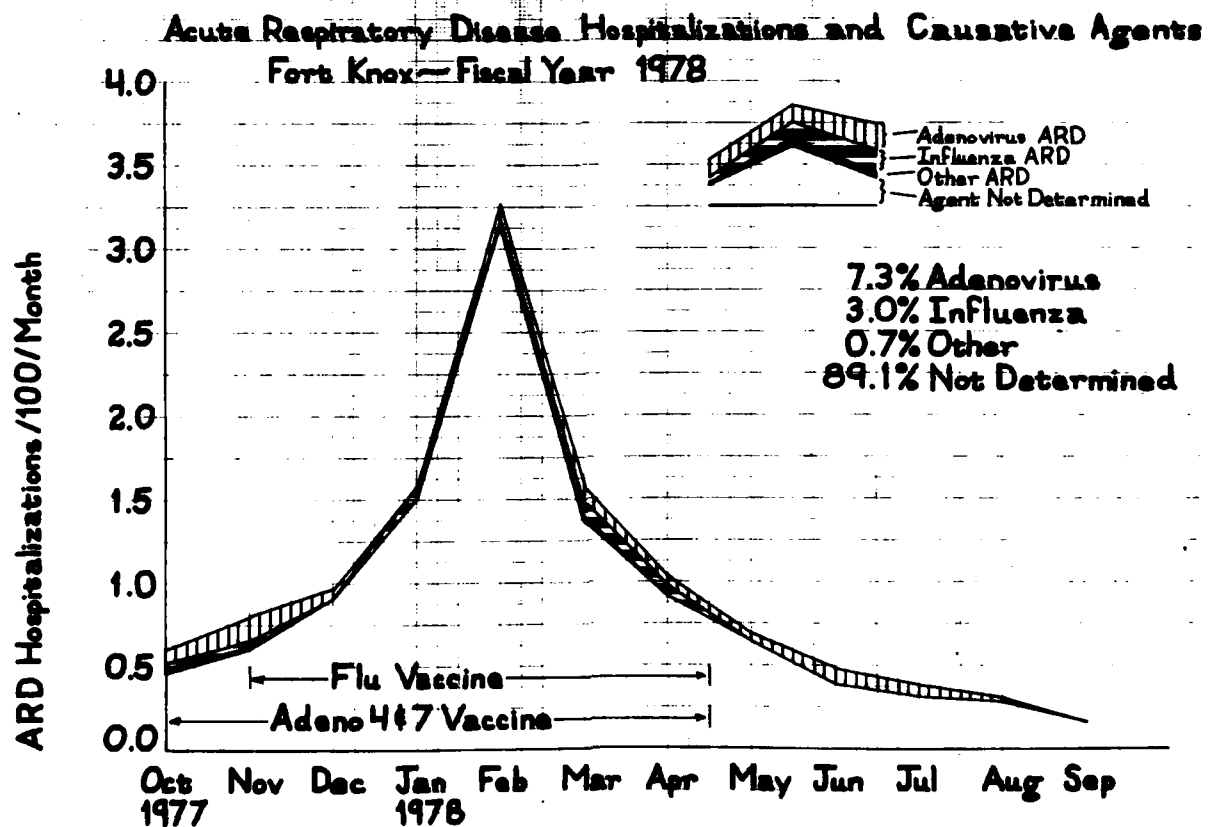
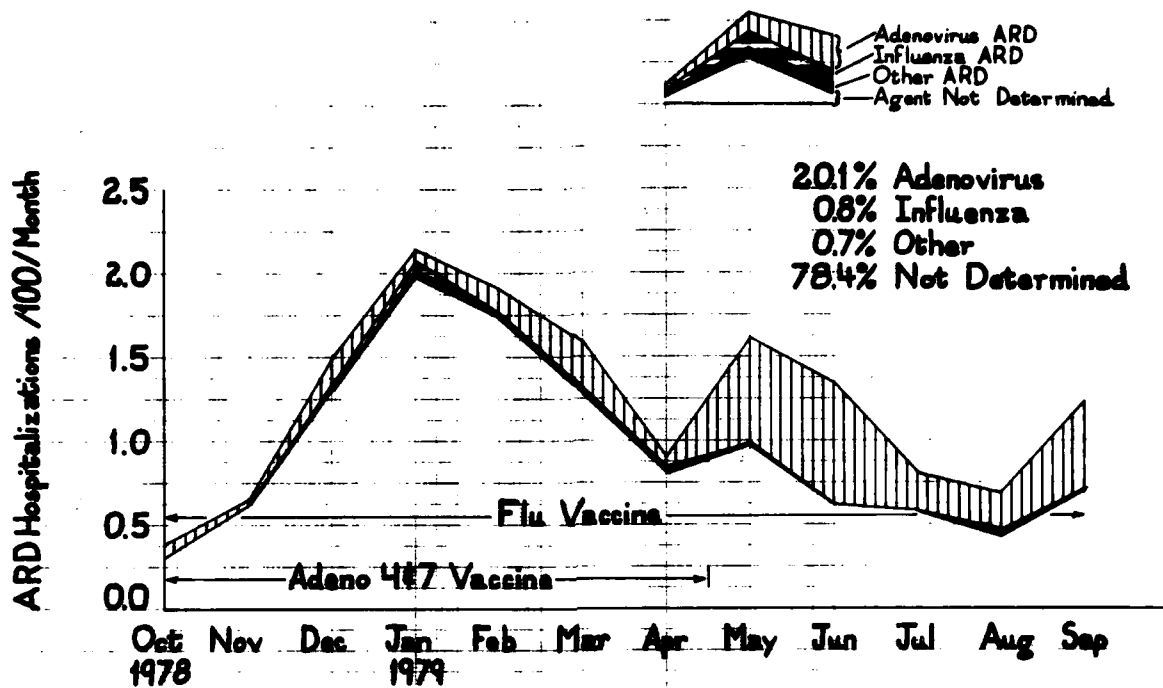
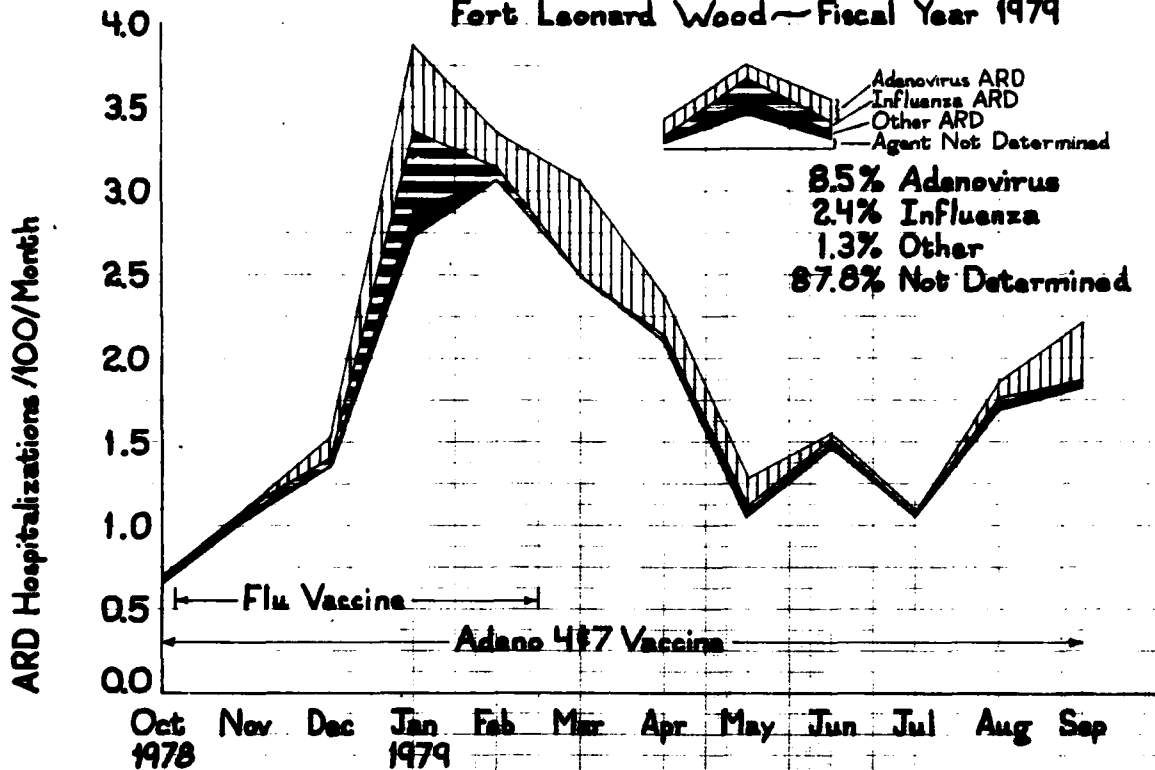
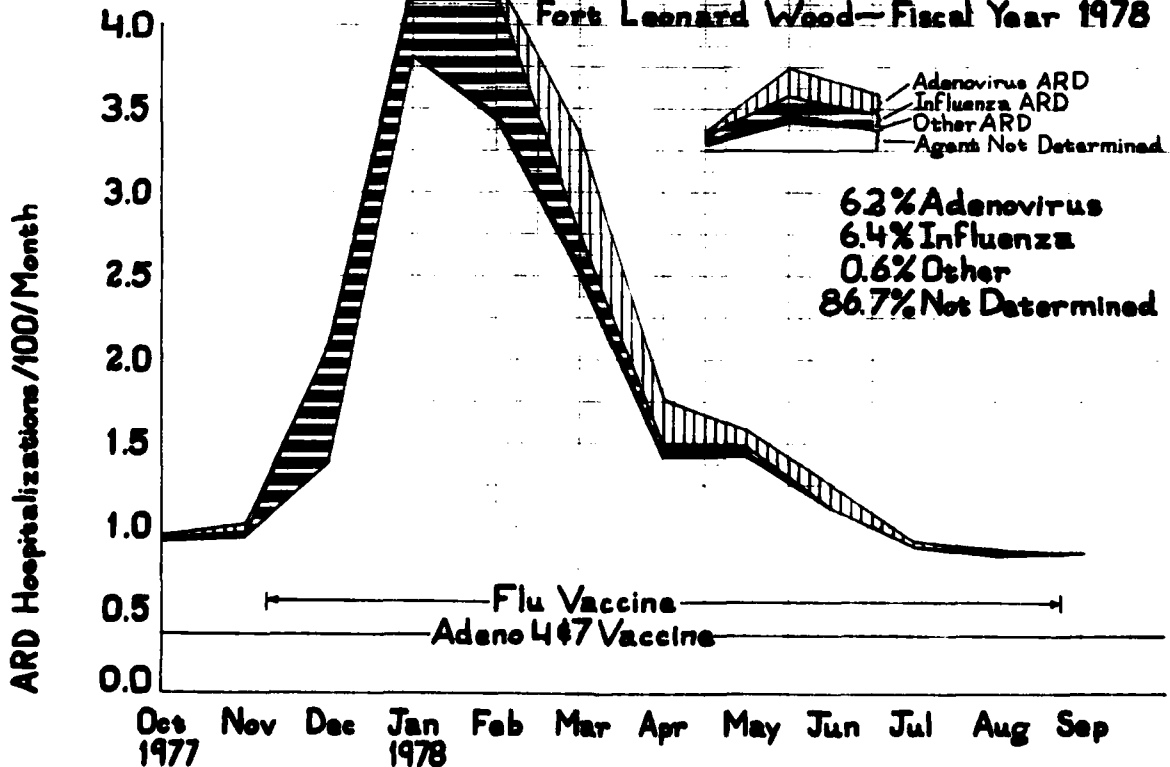


FIGURE 7

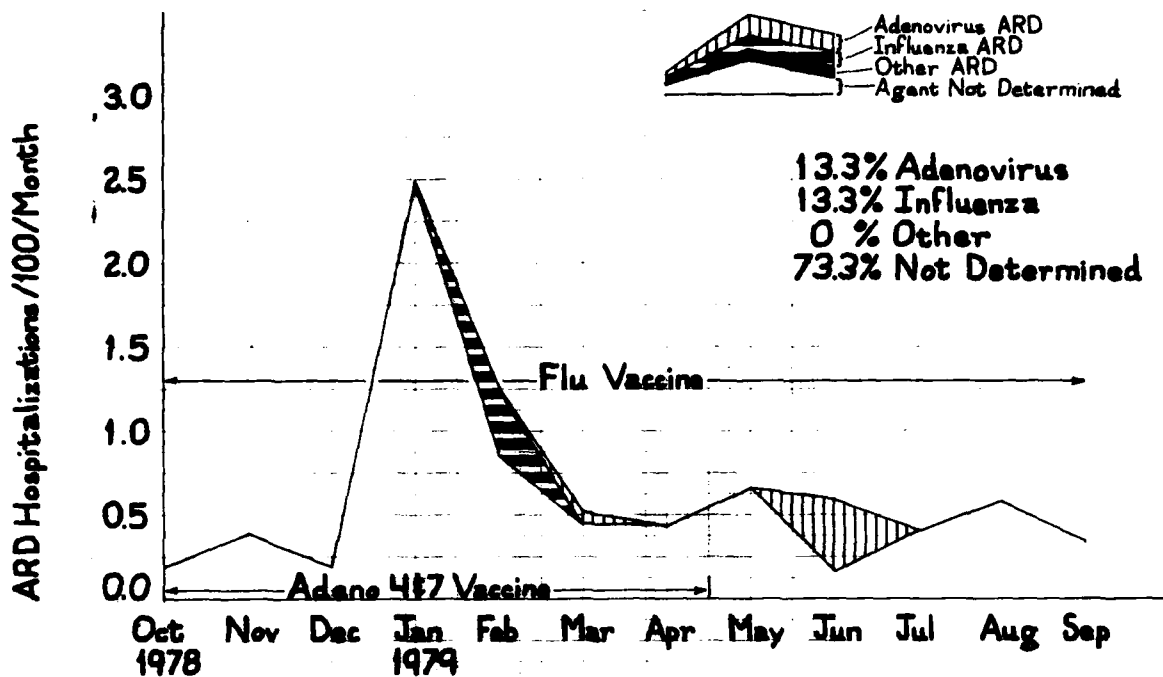
Acute Respiratory Disease Hospitalizations and Causative Agents  
Fort Leonard Wood - Fiscal Year 1979



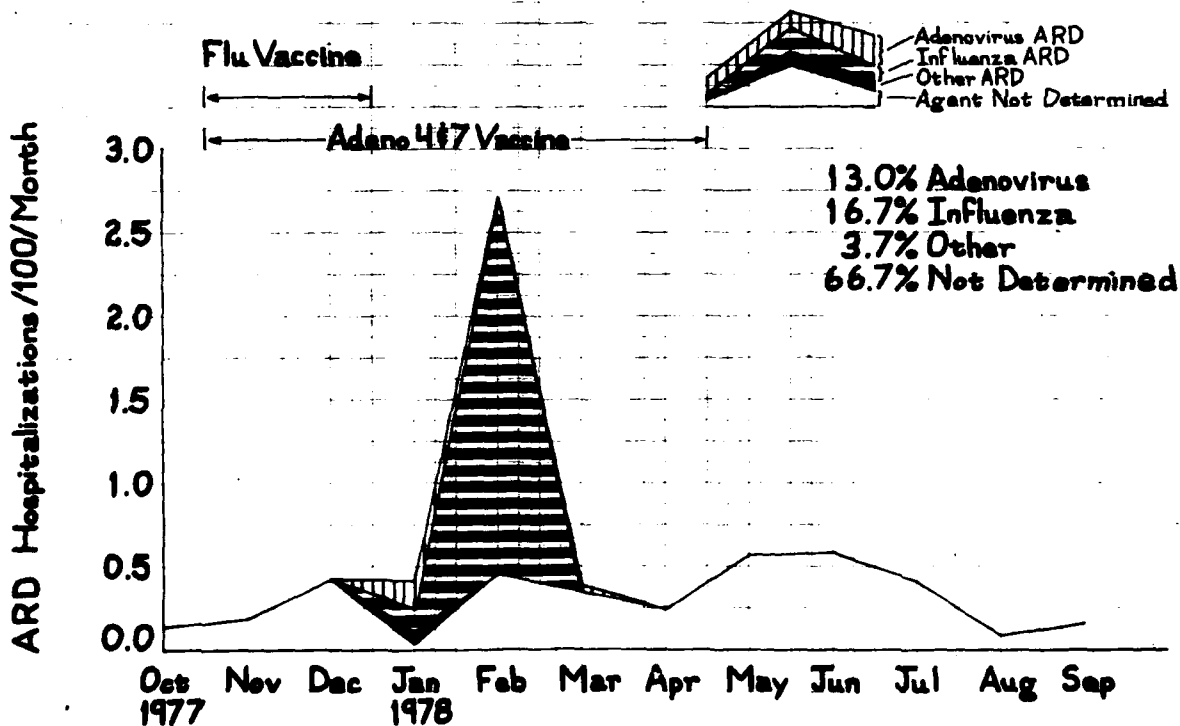
Acute Respiratory Disease Hospitalizations and Causative Agents  
Fort Leonard Wood - Fiscal Year 1978



**FIGURE 8**  
**Acute Respiratory Disease Hospitalizations and Causative Agents**  
**Fort Sill—Fiscal Year 1979**

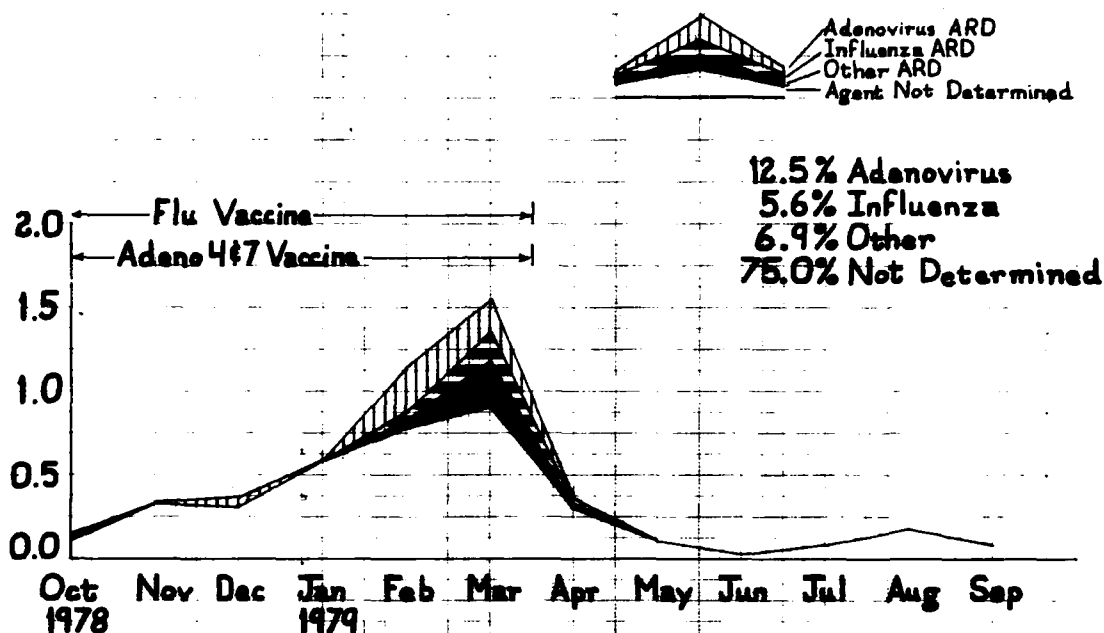


**Acute Respiratory Disease Hospitalizations and Causative Agents**  
**Fort Sill—Fiscal Year 1978**



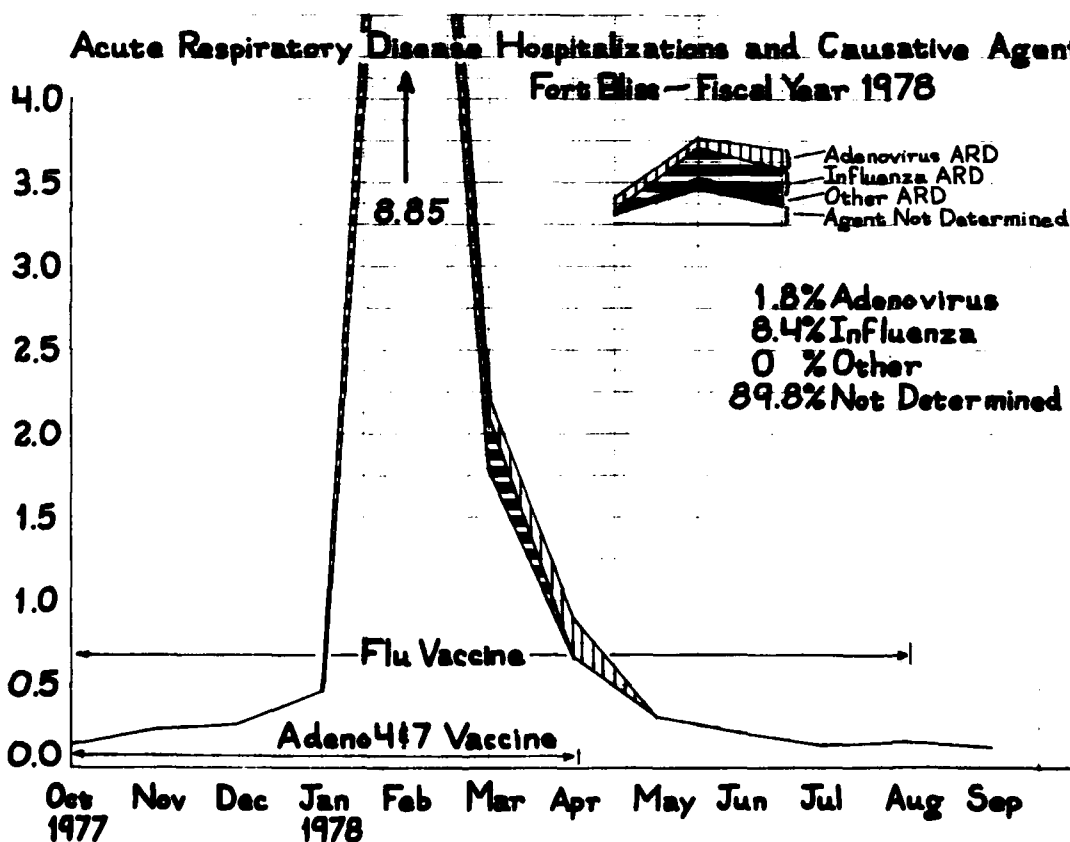
**FIGURE 9**  
**Acute Respiratory Disease Hospitalizations and Causative Agents**  
**Fort Bliss — Fiscal Year 1979**

ARD Hospitalizations /100/Month

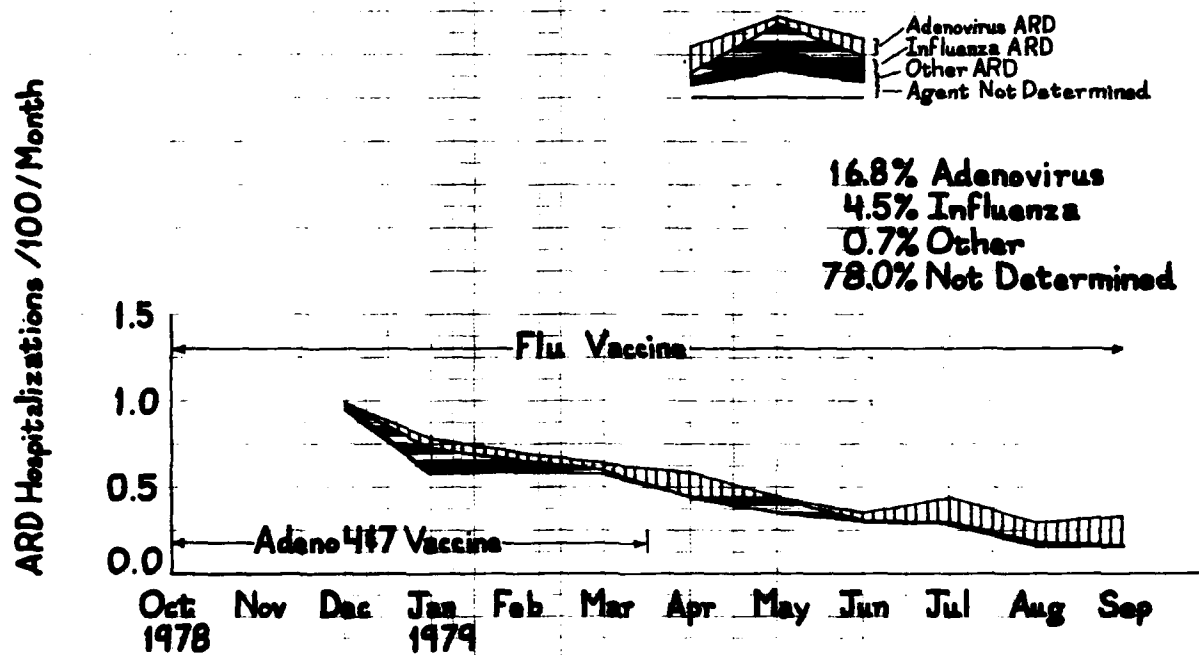


**Acute Respiratory Disease Hospitalizations and Causative Agents**  
**Fort Bliss — Fiscal Year 1978**

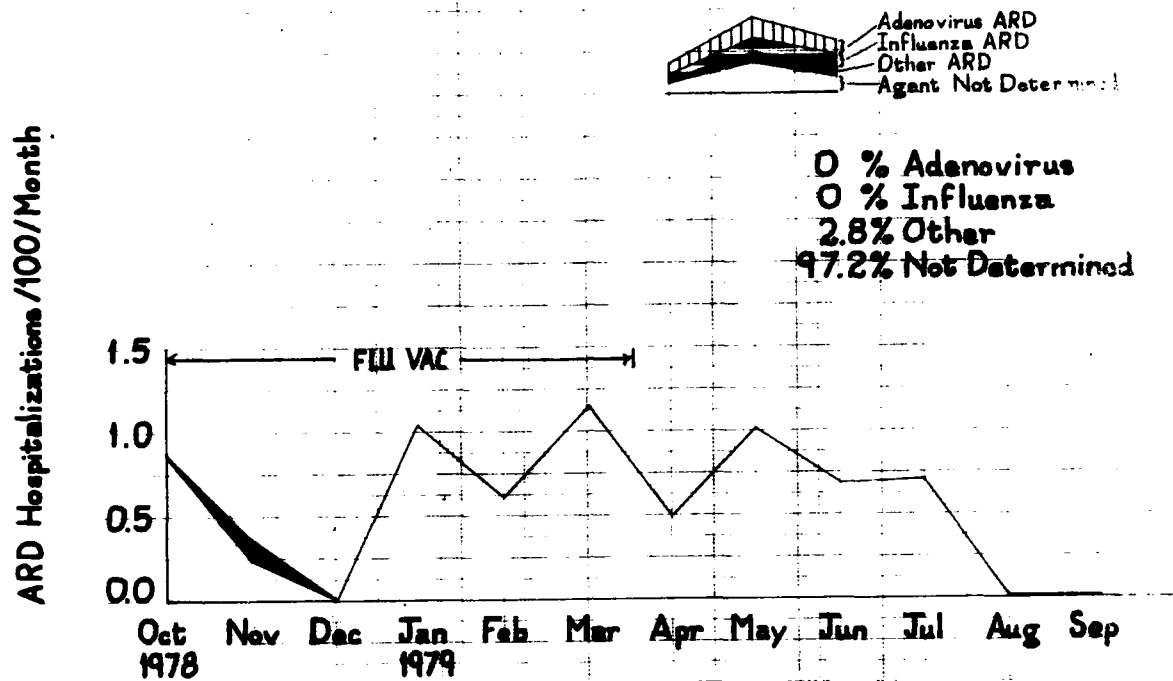
ARD Hospitalizations /100/Month



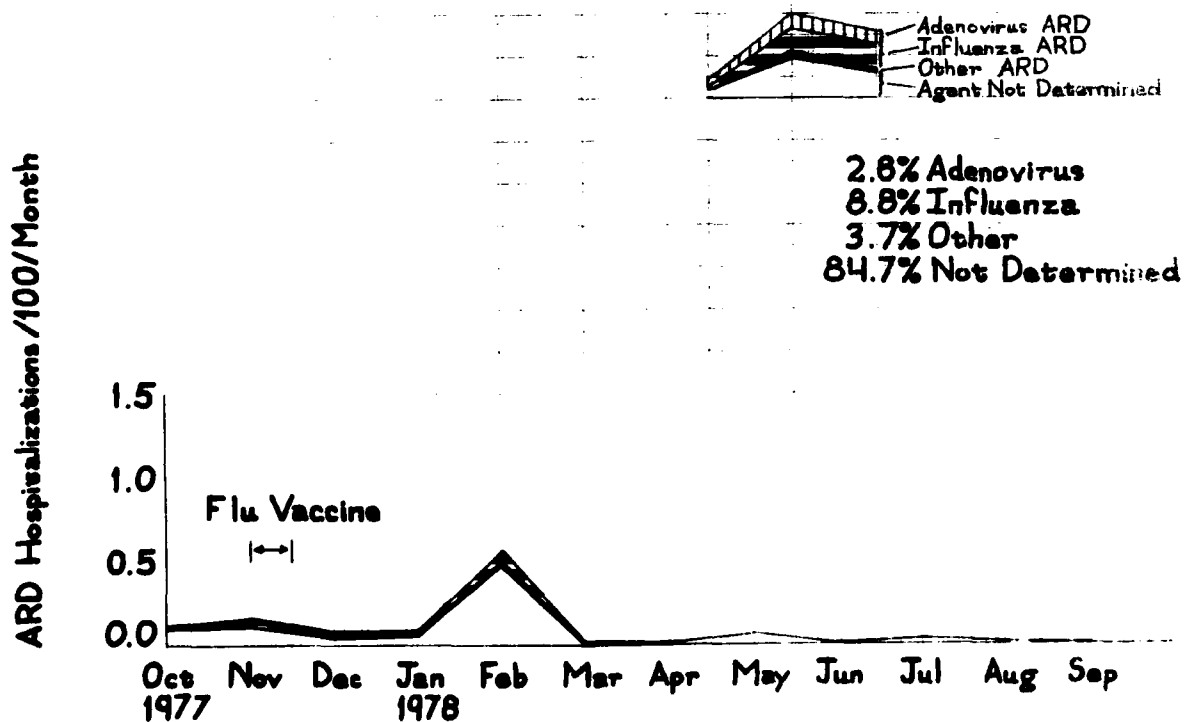
**FIGURE 10**  
**Acute Respiratory Disease Hospitalizations and Causative Agents**  
**Fort Benning — Fiscal Year 1979**



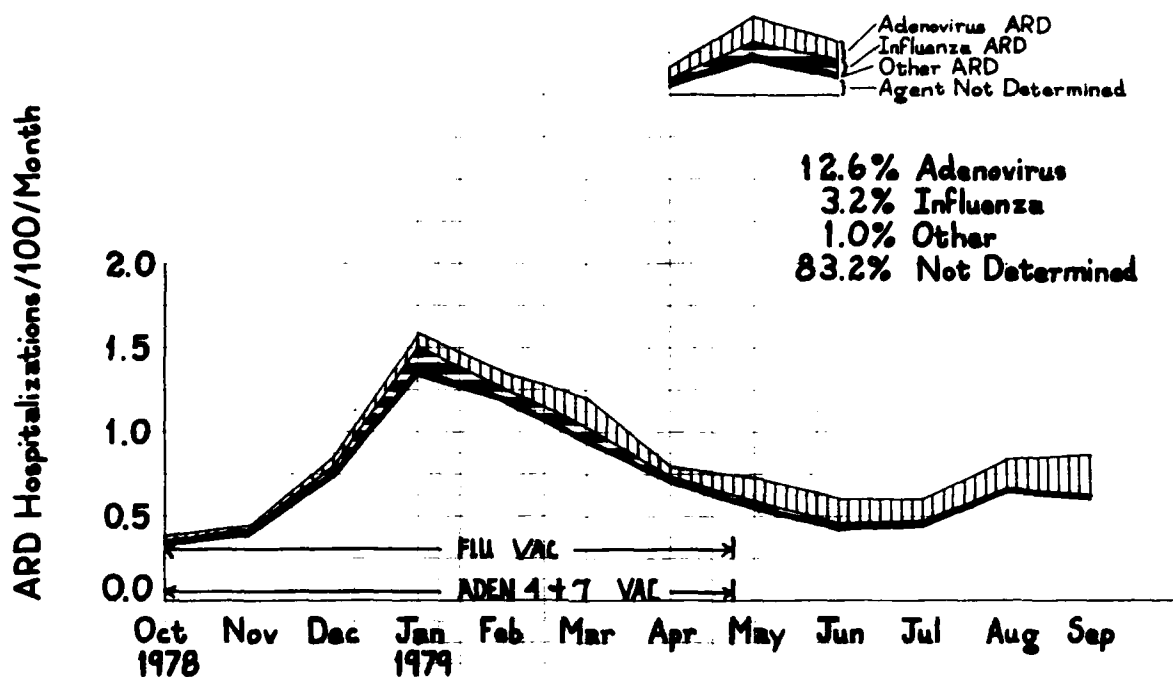
**FIGURE II**  
**Acute Respiratory Disease Hospitalizations and Causative Agents**  
**Fort Ord—Fiscal Year 1979**



**Acute Respiratory Disease Hospitalizations and Causative Agents**  
**Fort Ord—Fiscal Year 1978**



**FIGURE 12**  
**Acute Respiratory Disease Hospitalizations and Causative Agents**  
**All Stations—Fiscal Year 1979**



**Acute Respiratory Disease Hospitalizations and Causative Agents**  
**All Stations—Fiscal Year 1978**

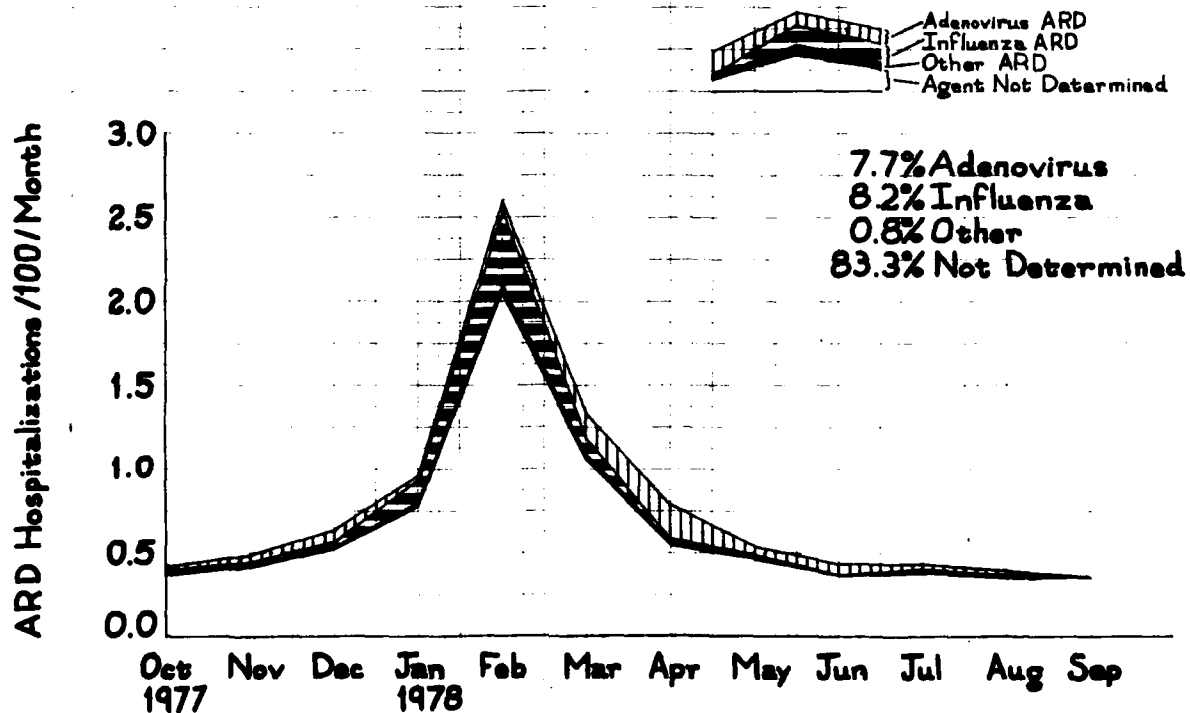


Table 1. Causative Agents of ARD, Isolations and Seroconversions  
FY: 1 OCT 78 - 30 SEPT 79

STATION	TOTAL	ISOLATIONS								TOTAL	SEROCONVERSIONS					
	ISOL SPEC	Ad 3	Ad 4	Ad 7	Ad 21	FLU A B	POLIO POOL	HSV	OTHER	PAIRS RECD.	ADENOVIRUS <1WK >2WK	FLU A <1WK >2WK	FLU B <1WK >2WK	MYCO PNEU		
FT DIX	867		53	6	49	4 (H <sub>1</sub> N <sub>1</sub> )	14	4	1 (Echo 9)	586	17 64	31 25	10 5	9		
FT JACKSON	565		3	1	3		7			220	10 9	77 4	16	3		
FT GORDON	446		3	11	10	1 (H <sub>1</sub> N <sub>1</sub> )	24			356	12 20	46 40	11 9	4		
McCLELLAN	106			1			2			38		5 17		1		
FT KNOX	1005	20	42		107	1 (H <sub>1</sub> N <sub>1</sub> )	16	2		298	10 77	10 6	2 1	5		
FT WOOD	918		16	1	41		14	2		694	14 34	32 18	12 3	12		
FT SILL	45		2		4	1 (H <sub>1</sub> N <sub>1</sub> )	4			26	3	4 5	3 1			
FT BLISS	63		2	1	1	1 (H <sub>1</sub> N <sub>1</sub> )	4		1 (CoxA-9)	58	1 3	2 3	1			
FT BENNING	264		2		36		19			79	3 9	12 9	1 1	1		
FT ORD (AIT)	37						17									
NTC (San Diego)	121			1			3	4		0						
ABERDEEN	12					11 (H <sub>1</sub> N <sub>1</sub> )				10		7				
FT BELVOIR	22					14 (H <sub>1</sub> N <sub>1</sub> )				6		2				
FT DEVENS	14					10 (H <sub>1</sub> N <sub>1</sub> )				10		1				
FT EUSTIS	3					2 (1-H <sub>1</sub> N <sub>1</sub> ) 2 (1-H <sub>3</sub> N <sub>2</sub> )				2	1					
FT MEAD	6					2 (H <sub>1</sub> N <sub>1</sub> )				0						
NSA FT MEAD	1					1 (H <sub>1</sub> N <sub>1</sub> )				0						
NAVAL REGIONAL MED. CTR. IL.	1					1 (H <sub>3</sub> N <sub>2</sub> )				0						
FT CARSON	18				1					4			2			
TRIPLER	18					1 (H <sub>1</sub> N <sub>1</sub> )				0						
LETTERMAN	3									0						
TOTALS	4536	20	123	22	252	50	107	12	2	2404	70 218	219 137	57 20	36		

% 4.8 29.5 5.3 60.4

87.3 12.7

% LAST YEAR 12.2 13.4 74.4

89 11

# ANTIGENIC EVALUATION OF ADENOVIRUS VACCINES FY 1979

## DESIGN:

Types 4 and 7 live enteric-coated Adenovirus vaccines containing  $10^{5.1}$  and  $10^{6.1}$  logs TCID<sub>50</sub> of virus were evaluated for immunogenicity during the year on the first recruits who received new vaccine lots distributed to Forts Knox, Dix, Jackson, Sill, Mc Clellan, and Wood. No sera were received from Forts Bliss or Benning. Serum neutralizing antibody titers were determined at this laboratory on 398 serum pairs consisting of pre-vaccine and 3 weeks post-vaccine samples. The microtiter homologous neutralization test which is routinely used in this laboratory was employed.

TABLE 1

	<u>Vaccine Lot #</u>	<u>Pairs Tested</u>	<u>Conversions/Susceptibles</u>	<u>Percent Conversions</u>
<u>FORT SILL</u>				
Adenovirus 4	7901	24	12/16	75.0
Adenovirus 7	8001	24	4/7	57.1
<u>FORT KNOX</u>				
Adenovirus 4	7701	25	12/17	70.6
Adenovirus 7	7801	25	4/9	44.4
<u>FORT WOOD</u>				
Adenovirus 4	7901	21	11/13	84.6
Adenovirus 7	8001	21	4/4	100.0
<u>FORT MC CLELLAN</u>				
Adenovirus 4	7701	19	9/11	81.8
Adenovirus 7	8001	19	5/5	100.0
<u>FORT JACKSON</u>				
Adenovirus 4	7701	50	28/33	84.9
Adenovirus 7	7801	50	18/23	78.3
<u>FORT DIX</u>				
Adenovirus 4	7701	60	20/51	39.2
Adenovirus 7	7801	60	28/30	93.3

## ACCUMULATIVE RESULTS

		<u>Total Pairs Tested</u>	<u>Conversions/Susceptibles</u>	<u>Percent Conversions</u>
Adenovirus 4	Vaccine	199	92/141	65.3
Adenovirus 7	Vaccine	199	63/78	80.8
% Susceptible to Adenovirus 4		=	141/199	= 70.9%
% Susceptible to Adenovirus 7		=	78/199	= 39.2%

# INVESTIGATION TO DETERMINE THE SPECIFIC TYPES OF ADENOVIRUS INFECTIONS BY MICROTITER SERUM NEUTRALIZATION TESTS

## INTRODUCTION

The Adenovirus Surveillance Program was established to monitor the presence of infectious agents of Acute Respiratory Disease (ARD) in Basic Combat Trainees (BCTs). Virus isolations and identifications in cell culture and the Complement Fixation (CF) test have been the primary procedures used in determining the etiological agents of ARD for the Program. The Adenovirus CF Antigen is used to detect the group specific antibodies associated with generalized Adenovirus infection (1). Although the CF test is an excellent screening procedure for detecting Adenovirus infection, it will not determine specific Adenovirus serotypes. It has been reported that the CF test is less sensitive than the neutralization and hemagglutination test, especially in children (2).

The Adenovirus Surveillance Programs' isolation and seroconversion ( $\geq 4$  fold CF titer) rates for Adenovirus and Influenza A for Fiscal Year 1978 was 7.6% and 14% respectively (3). Thus, a large number of causative agents of ARD were not identified. It is the intension of this study to determine if the Microtiter Serum Neutralization test is a sensitive test for differentiating between Adenovirus 4, 7, and 21 serotypes.

## MATERIALS AND METHODS

Serum neutralization tests will be performed as described by Lennette (3). A major modification to the procedure will be the conversion to a microtiter system. FL amnion cells purchased from ATCC and grown in Media L15 will be the cell substrate used in the system. Adenovirus types 4, 7 and 21 will serve as antigen sources. Viral titers that demonstrate 100 TCID<sub>50</sub> between 3-4 days after inoculation will be selected. Only those sera which do not elicit a four-fold rise in antibody titer to Adenovirus by CF and do not have an Adenovirus isolated from the throat culture will be excluded from the study. Cultures will be read daily and recorded when 32 to 320 TCID<sub>50</sub> are observed in the virus titration by the Reed and Meunch method. The highest serum dilution microtiter well in which there is no Adenovirus CPE will be considered the end point.

## RESULTS AND DISCUSSION

A total of 201 individuals was selected for the study. As demonstrated in Table 1, only nine isolates out of 68 specimens were recovered from individuals in the  $\leq 1$  week group and 72 isolates out of 133 specimens were recovered from the  $\geq 2$  week group. A total of seventy-seven

TABLE 1  
VIRUS ISOLATIONS

FORT	WEEK OF TRAINING	NO. PATIENTS	ISOLATES			PP	TOTAL	% POSITIVE OF ISOL RECOVERED
			4	7	21			
WOOD	<1	42	1	0	3	1	5	40.7
	≥2	40	4	3	21	0	28	40.7
JACKSON	<1	5	0	0	0	0	0	23.5
	≥2	30	4	4	11	0	19	23.5
GORDON	<1	6	1	0	2	0	3	18.5
	≥2	24	6	1	5	0	12	18.5
DIX	<1	11	0	0	1	0	1	6.2
	≥2	12	0	2	1	1	4	6.2
KNOX	<1	3	0	0	0	0	0	7.4
	≥2	17	1	1	4	0	6	7.4
ORD	<1	0	0	0	0	0	0	1.2
	≥2	5	0	1	0	0	1	1.2
SILL	<1	0	0	0	0	0	0	2.5
	≥2	5	0	0	0	2	2	2.5
BLISS	<1	1	0	0	0	0	0	0.0
	≥2	0	0	0	0	0	0	0.0
% POSITIVE OF TOTAL SPECIMENS								
SUB TOTAL:	<1	68	2	0	6	1	9	4.5
	≥2	133	15	12	42	3	72	35.8
GRAND TOTAL:		201	17	12	48	4	81	40.3

TABLE 2  
NEUTRALIZATION STUDIES FOR ADENOVIRUS 4, 7, 21  
PATIENTS WITH NO VIRUS ISOLATED

WEEK OF TRAINING	NO. TESTED	NO. RISES	SPECIFIC RISE TO ADENOVIRUS TYPE			RISES TO 2 SEROTYPES	RISES TO 3 SEROTYPES
<u>&lt;1</u>	59	4	4	7	21	17-11-(4& 7) 3-(4&21) 3-(7&21)	14
<u>&gt;2</u>	61	8	3	6	9	13- 1-(4& 7) 4-(4&21) 8-(7&21)	22

TABLE 3  
NEUTRALIZATION STUDIES FOR ADENOVIRUS 4, 7, 21  
PATIENTS WITH A VIRUS ISOLATED

WEEK OF TRAINING	ADENOVIRUS	TOTAL POS	SPEC RISE	NO RISE	RISE TO 2 ADENO	RISE TO 3 ADENO	NO RISE
<u>&lt;1</u>	4	2	0	0	1 (4& 7)	1	0
	7	0	0	0	0	0	0
	21	6	4	0	0	2	0
	Polio	1	0	0	1 (4& 7)	0	0
SUBTOTAL :		9	4	0	2	3	0
<u>&gt;2</u>	4	15	2	4	2 (4& 7)	6	1(21)
	7	12	3	2	4 3(4& 7) 1(7&21)	2	1(21)
	21	42	19	2	12 7(4&21) 5(7&21)	9	0
	Polio	3	0	0	2 (4&21) (4& 7)	1	0
SUBTOTAL :		72	24	8	20	18	2
TOTAL :		81	28	8	22	21	2

# INVESTIGATION TO DETERMINE THE SPECIFIC TYPES OF ADENOVIRUS INFECTIONS BY MICROTITER SERUM NEUTRALIZATION TESTS.

Adenoviruses and four Polioviruses were isolated from throat cultures. Adenovirus type 21 was the most common virus isolated (48) with Adenovirus type 4 (17) and 7 (12) constituting the remaining viruses.

Table 2 represents the results of the serum neutralization tests for 120 individuals who did not have any viruses isolated but did have four-fold or greater rises to Adenovirus by the CF test. Only 12 of the 120 individuals lacked four-fold rises in neutralizing antibodies to Adenovirus types 4, 7 or 21. A total of 42 out of the remaining group of 188 (38.9%) had a rise to only one serotype; whereas, 66 individuals (55.0%) had rises to two or all three serotypes tested.

Table 3 provides information on 81 individuals who had viruses isolated from throat cultures and had a four-fold rise in CF Antibody titers to Adenovirus. Serum neutralization tests to Adenovirus 4, 7 and 21 indicated that eight individuals did not have any Adenovirus neutralizing antibodies, 28 had rises to only one serotype and 43 had rises to two or all three serotypes. There were two individuals who had Adenovirus type 4 or 7 isolated but had a  $\geq$  four-fold rise to Adenovirus type 21.

Since all inductees are required to receive Adenovirus 4 and 7 vaccines as they enter the service, all four-fold or greater rises to Adenovirus 4 and 7 detected in the  $\leq$  one week of training group must be assumed to be associated with a vaccine response. The exception would be individuals in which an Adenovirus type 4 or 7 isolate was recovered. Adenovirus type 21 appears to be causing URI in the first week group as evidenced by the six isolates recovered and serologic rises in 33/68 individuals.

The large number of multiple rises found in the  $\geq 2$  week of training group is difficult to assess. The presence of cross serum neutralization reactions among Adenovirus has been well documented (4, 5, 6). It has also been reported that heterotypic rises in antibodies to Adenovirus may occur with both the hemagglutination-inhibition and neutralization tests in as high as 25.0% of adult populations (7). The multiple rises observed in this study group (73/133-54.9%) indicate that heterotypic responses to Adenovirus 4, 7 and 21 were very high. Several possibilities may be responsible for this high rate of multiple rises: (1) infection with Adenovirus 4, 7 or 21 resulting in heterotypic responses which were enhanced by the recent immunization with the Adenovirus vaccines (2) 78/133 of these individuals were in their second or third week of training and were therefore responding to the Adenovirus vaccines.

Adenovirus type 21 appeared to be a significant cause of URI in the  $\geq$  two week of training group. This was supported by the 42 Adenovirus 21 isolates recovered, 30 specific serologic rises and 66 individuals who

## INVESTIGATION TO DETERMINE THE SPECIFIC TYPES OF ADENOVIRUS INFECTIONS BY MICROTITER SERUM NEUTRALIZATION TESTS.

had multiple rises in which one rise was to Adenovirus type 21. In addition a comparison of the ratio of geometric mean titer (C/A) resulted in a ratio of 3.77 for the  $\leq 1$  week group to 7.44 for the  $\geq 2$  week of training group.

### SUMMARY

The neutralization test is capable of detecting specific antibody which can be correlated with protection against disease caused by specific Adenovirus types. Heterotypic responses to Adenovirus infection has been reported with both the hemagglutination inhibition and neutralization tests. The results of this study indicate that heterotypic reactions were very high. The fact that all individuals entering basic training receive the Adenovirus types 4 and 7 vaccines presented some unusual problems. Serologic rises observed for Adenovirus 4 and 7 are particularly difficult to assess in the absence of an isolate. The recent immunization with the Adenovirus vaccines appeared to have primed individuals to produce a large number of heterotypic rises (73/133) to Adenovirus. This observation is supported by Jackson who reported that rises in heterotypic neutralizing antibody appears more frequently if heterotypic antibodies are present at the time of infection (5).

It is concluded that in evaluating Basic Combat Trainees for Adenovirus infection, the neutralization test cannot be solely used to establish the cause of infection.

### REFERENCES

1. Top, F.H. 1976. Adenoviruses, P 448-451. In N.R. Rose and H. Friedman (ed) Manual of Clinical Immunology, American Society of Microbiologist, Washington, D.C.
2. Schmidt, N. M., E. H. Lennette, and C. J. King. 1966. Neutralizing, Hemagglutination - Inhibiting and Group Complement - Fixing Antibody Responses in Human Adenovirus Infections, J. Immunology. 97: 64-74.
3. Rose, H. M. 1969. Adenoviruses, P 205-226. In E. H. Lennette and N. J. Schmidt (ed.) Diagnostic Procedures for Viral and Rickettsial Diseases. American Public Health Association, Inc., N. Y.
4. Kasel, J. A., Alling, D. W., Glade, P. R., Banks, P. A. Heterotypic Antibody Responses in Man Following Vaccination with Adenovirus Type 1 Hexon Antigen. Proc. Soc. Exp. Biol. Med. 126: 895-896, 1967.

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INFECTIONS BY MICROTITER SERUM NEUTRALIZATION TESTS.

REFERENCES (Contd.)

5. Jackson, G. G., R. E. Muldoon, Viruses Causing Common Respiratory Infection in Man. IV Reoviruses and Adenoviruses. 1973 Vol. 128 No. 6, 811-866.
6. Selivanov, A. A., Kovaleva, T. P., Smorodintsev, A. N. Specific Humoral Immunity Among Volunteers With Experimental Adenovirus Infection. Arch. Gesamte Virusforsch. 36: 36-42 1972.
7. Rosen, L. 1961 Hemagglutination - Inhibition Antibody Responses In Human Adenovirus Infections, Proc. Soc. Exp. Biol. Med. 108: 474-479.
8. Smith, Creed D. et al; Prophylactic Methods in the Prevention of Disease Among Army Personnel. Annual Report DA Project - 3 A061102 871 Q December 1978.

## VIRAL SENSITIVITY OF A TRANSFORMED HUMAN EMBRYONIC KIDNEY CELL LINE (HEK-T)

### INTRODUCTION

In 1966 the Army initiated an Acute Respiratory Disease (ARD) surveillance program for all basic training centers (1). The Complement Fixation (CF) test for antibody determinations and cell culture virus isolation were the primary procedures used for the study. Basic cell cultures for virus isolation and identification utilized were the Human Embryonic Kidney (HEK), Primary Rhesus Monkey Kidney (RMK), Human Diploid Fibroblast (IMR-90), and some continuous cell lines. The HEK cells have been in wide use and have been the most sensitive for Adenovirus isolations (2,3). With the diminishing supply of primary HEK cells from commercial sources, a replacement for these cells is needed. A potential candidate is the Adenovirus type 5 DNA transformed human cell line (HEK-T) (4). Many other continuous cell lines are capable of supporting the growth of Adenovirus, but none have approached the sensitivity and consistency of primary HEK cells. In this trial study, the HEK-T was compared with current cell cultures used in the Adenovirus Surveillance and Clinical Virology sections of this laboratory. All cell cultures were rated for their ability to recover viruses, morphologic characteristics, longevity, and for clarity of cytopathic effect (CPE).

### MATERIALS AND METHODS

The inocula were throat swabs from the Adenovirus Surveillance and viral specimens from Clinical Virology. The HEK-T cell line was received at the 71st passage from Walter Reed Army Institute of Research (WRAIR). The cells were grown in T-15 plastic flasks containing 25 ml of L-15 supplemented with 10% fetal calf serum, 50 mg gentamicin/ml and 5 mg fungizone/ml. Cells were passed by using 0.05% trypsin and 0.025% versene and were split every 4 days at a ratio of 1:5. Cell culture tubes (16 X 125 mm) were seeded at the concentration of  $1.0-1.5 \times 10^5$  cells/ml in L-15 with 2.0% fetal calf serum. The HEK-T cell line was included with current cell cultures used in both sections. Virus isolation and identification were performed using standard procedures (5).

### RESULTS AND DISCUSSION

A total of 990 viral specimens was studied from April - October 1978. In the Adenovirus Surveillance Section 76 isolates were recovered with Adenovirus (Type 4, 7 and 21) comprising 65.8% (50/76) of the total as demonstrated in Table 1. HEK cells proved to be the most sensitive for the Adenoviruses with an isolation rate of 51.3% (39/76), followed by HEK-T with an isolation rate of 21.1% (16/76). There were little differences in the recovery of Herpes simplex, Polio and Influenza viruses by RMK, IMR-90, and HEK-T cell cultures.

TABLE 1

A COMPARISON OF THE SENSITIVITY OF A TRANSFORMED  
HUMAN EMBRYONIC KIDNEY CELL (HEK-T) WITH OTHER CELL LINES

ADENOVIRUS SURVEILLANCE		# OF ISOLATES	HEK	RMK	IMR- 90	*VMK	*FL	HEK-T	TOTAL # SPEC TESTED	% POS
Adeno	4	20	16	0	1	-	-	5		
	7	9	4	0	1	-	-	5		
	21	21	19	0	0	-	-	6		
Herpes		7	4	0	4	-	-	3		
Polio		18	2	8	5	-	-	9		
Influenza A		1	1	0	0	-	-	0		
SUB TOTAL:		76	46	8	11	-	-	28	642	11.8
CLINICAL SPEC										
Adeno	2	1	1	0	0	0	0	0		
	3	1	1	0	0	0	0	0		
	19	2	2	1	0	0	0	0		
Coxsackie	A 9	1	1	1	1	1	0	0		
	A16	1	0	1	0	1	0	0		
	B 2	1	0	1	0	1	0	0		
	B 3	2	0	2	0	3	2	2		
	B 4	2	0	2	0	2	0	0		
Herpes		26	15	5	11	26	21	1		
Polio		3	1	0	0	1	1	0		
CMV		1	0	0	1	0	0	0		
Mumps		1	1	0	0	0	0	0		
Echo		7	1	1	1	1	0	0		
Echo		9	1	1	1	2	0	0		
Echo		11	1	1	1	1	0	0		
SUB TOTAL:		43	25	16	16	39	24	3	348	12.1
GRAND TOTAL:		119	71	24	27	39	24	31	990	11.9

\* Cells not tested for Adenovirus Surveillance specimens.

#### VIRAL SENSITIVITY OF A TRANSFORMED HUMAN EMBRYONIC KIDNEY CELL LINE (HEK-T)

In the Clinical Virology Section, Herpes simplex virus was isolated most frequently at a rate of 60.5% (26/43), followed by Picornavirus group 23.3% (10/43), and Adenovirus 9.3% (4/43) as shown in Table 1. The majority of these viral specimens were from patients with a diagnosis of genital infections. In this section, the most sensitive cell culture for Herpes simplex virus was Vero Monkey Kidney (VMK). The other three viruses (2 Coxsackie B-3 and 1 Herpes simplex viruses) were isolated in the HEK-T cell line, Adenovirus was not isolated at all in this cell line.

The total virus isolation rate for both sections was 12.0% (119/990) for all cell culture systems. Primary HEK cells appeared to be the most sensitive cell cultures for virus isolation 59.7% (71/119), followed by VMK 32.8% (39/119), and HEK-T 26.1% (31/119). HEK cells proved to be the most sensitive for Adenovirus isolation with a rate of 36.1% (43/119). The Adenovirus isolation rate for HEK-T cell line was 13.4% (16/119), and IMR-90 cell line 1.7% (2/119) as shown in Table 1.

The growth of transformed human cells was more consistent in T-75 plastic flasks than in glass culture tubes (16 X 125 mm). The cell morphology tended to slough off the glass wall of the tube much faster than in a plastic flask. There was no difference in the methodology in seeding these cells in glass tubes or plastic flasks.

All cell culture tubes are normally placed in roller drums for 10-14 days before they are tested for hemadsorption. Cell degeneration in the transformed human cell line occurred rapidly (4-6 days) in the roller drum as well as in a stationary position. This rapid cell degeneration in HEK-T cells made it very difficult to differentiate between the presence of a slow growing Adenovirus from normal cell degeneration.

#### SUMMARY

The Adenovirus type 5 DNA transformed human cell line (HEK-T) was compared with currently used cell cultures in the Adenovirus Surveillance and Clinical Virology sections of this laboratory. Overall HEK cells were the most sensitive cell culture for most virus isolations. HEK-T cells appeared to lack the sensitivity for viral isolation in both sections. This was particularly true when comparing HEK-T to HEK cells. The problems with cellular degeneration (4-6 days) and the sloughing of the monolayer were particularly troublesome. It was very difficult to differentiate between the presence of cytopathogenic effect due to viral activity from normal cell degeneration.

**VIRAL SENSITIVITY OF A TRANSFORMED HUMAN  
EMBRYONIC KIDNEY CELL LINE (HEK-T)**

A lower passage level of HEK-T cells has been received from WRAIR, and plans are being made to evaluate these cells further.

**REFERENCES**

1. Dudding B.A., Top F.A., Winter, PE et al, Acute Respiratory Disease in Military Trainees - The Adenovirus Surveillance Program 1966-1971. Am. J. of Epidemiology 97: 187-198, 1973.
2. Fox J.P., Hall C.E., Cooney M.K., The Seattle Virus Watch. VII. Am. J. of Epidemiology 105: 362-386, 1977.
3. Jackson G.G., Muldoon R.L., Viruses Causing Common Respiratory Infections in Man. IV. Reoviruses and Adenoviruses. 128: 811-876, 1973.
4. Graham F.L., Smiley J.S., Russel W.C., Nairn R., Characteristics of a Human Cell line Transformed by DNA from Human Adenovirus Type 5. J. Gen. Virology 36: 59-74, 1977.
5. Rose H.M.: Adenovirus. Diagnostic Procedures for Viral and Rickettsial Infections. 4th Edition. New York, Am. Public Health Assoc. 1969, PP 205-206.

PENICILLINASE-PRODUCING NEISSERIA GONORRHOEAE/  
NGU SURVEILLANCE

INTRODUCTION

In early 1976 the first case of penicillinase-producing Neisseria gonorrhoeae (PPNG) was reported in Maryland in a member of the U.S. Air Force whose last duty assignment was in the Philippines (1). Since that time, numerous cases have been reported in other returnees from the Far East area and their primary contacts. Over 26 states have become involved with these cases with the majority of PPNG isolates (>51%) being found in California alone (2). In this study, all Neisseria gonorrhoeae (GC) isolates were tested for sensitivity to penicillin, and those found resistant were further assayed for beta-lactimase production.

MATERIALS AND METHODS

Two major groups of personnel were surveyed for PPNG: 1. Those military personnel undergoing separation physicals whose last tour of duty was in Korea, Japan, Hawaii, the Philippines, or other Pacific stations and who either exhibited gonorrheal symptoms or expressed fear of contact, and 2. San Francisco Bay Area personnel who demonstrated failure of cure with penicillin therapy after 3-7 days. Personnel terminating service were cultured on Transgrow medium at the Oakland Army Base clinic and sent to the Department of Pathology Area Laboratory Service, Letterman Army Medical Center, for culture and sensitivity. Bay Area failure of cure cases were sampled by the Preventive Medicine Section and forwarded to Department of Pathology Area Laboratory Service for culture and sensitivity.

Cultures received in the laboratory were tested for gram reaction and morphology and, regardless of results, a 20% sucrose solution was inoculated for the isolation of possible L-forms. Only Gram negative diplococci were identified and tested for sensitivity to penicillin. Those Neisseria sp. showing resistance (<20mm) to the 10 mg penicillin disc were further assayed for the enzyme beta-lactimase (penicillinase).

At the time of original culturing, the patient was interviewed for a personal history, giving such information as sex, race, last duty station, prior treatment for gonorrhea (if any) and, if they were undergoing a separation physical, a forwarding address for follow-up purposes was obtained.

Starting 1 March 1978 testing for Chlamydiae sp. was initiated. Second cultures were secured on all personnel along with serum specimens. The second culture was taken with a calginate swab on metal stems and submitted in a slightly modified tissue culture medium. This second culture and the serum was forwarded to the laboratory of Dr. Julius Schachter, University of California Medical Center, San Francisco, for isolation and identification of any Chlamydiae sp. and for titering for anti-Chlamydiae antibodies.

PENICILLINASE-PRODUCING NEISSERIA GONORRHOEAE/  
NGU SURVEILLANCE

RESULTS AND DISCUSSION

Over a 13 month period 55 cultures were taken for PPNG study purposes, and of these 48 were also analyzed for Chlamydiae involvement (see summary). There were 13 positive GC isolates and of these 12 were sensitive to penicillin with one being resistant. This penicillin resistant isolate proved to be negative for beta-lactimase production.

All of the positive GC cultures were from male personnel. All were symptomatic. 7 of these positive cultures were from Korea, 5 from the Bay Area, and 1 was from Georgia. There were no L-forms isolated.

Of those 48 specimens submitted for which Chlamydiae isolation was attempted all were negative. Serological evidence as determined by IgM and IgG MicroImmunoFluorescence(MIF) titration indicated 30 cases of either active infection (7 individuals having IgM titers greater than or equal to 1:32) or past infection (23 individuals having IgG titers of greater than or equal to 1:64 with accompanying IgM titers of less than 1:32).

SUMMARY

Over a 13 month period 55 individuals were screened for PPNG and 48 for Chlamydiae, both those arriving from the Far East and local Bay Area permanent and visiting personnel. There were no PPNG isolated and no Chlamydiae confirmed by isolation. Of the 13 GC isolates, only 1 proved to be resistant to penicillin and was beta-lactimase negative. There was serological evidence for 7 active and 23 past Chlamydiae infections.

SUMMARY OF SURVEY RESULTS OF PPNG/NGU STUDY  
FROM: 1 December 1978 to 31 December 1979.

Sex: Male - 54  
Female - 1

Race: Caucasian - 20  
Negro - 28  
Oriental - 3  
Unknown - 4

Last or Current Duty Station: Japan - 0  
Korea - 33  
Hawaii - 5  
Bay Area - 13  
Other - 4

SUMMARY OF SURVEY RESULTS OF PPNG/NGU STUDY(Cont'd.)  
FROM: 1 December 1978 to 31 December 1979.

Culture Results:	<u>PPNG</u>	<u>Chlamydiae</u>
Negative:	55	48
Positive:	0	0

Chlamydiae Serology Results:

Negative:	18
Active infection:	7
Past infection:	23

GC Penicillin Sensitivity:

Sensitivity:	12
Resistant:	1

Resistant Culture Assay for beta-lactimase:

Positive:	0
Negative:	1

Number Positive L-form isolates: 0

Total Cultures Tested:

PPNG:	55
Chlamydiae:	48

REFERENCES

1. Culliton, B.J. Science, 194: 1395-1397, 1976.
2. Siegel, M.S., et. al. Journal of Infectious Diseases, 137: 170-175, 1978.

## MENINGOCOCCAL CARRIER SURVEILLANCE

### INTRODUCTION

Meningococcal carrier surveys were performed at Fort Ord at monthly intervals in order to monitor frequency of carriers and distribution of serogroups among personnel stationed there.

### MATERIALS AND METHODS

Various messhalls on post were used as collection points in order to sample a large number of individuals in a short time. No attempt at selection was made; the first 100 volunteers were sampled. Retro-uvula swabs were obtained and streaked directly onto Columbia Chocolate Agar (CCA) plates containing 5% sheep blood, 1% Isovitalex and inhibitors (6 mcg/ml lincomycin and 25 units/ml polymixin B). The plates were incubated overnight at 37°C in a 5-10% CO<sub>2</sub> atmosphere and then transported to this laboratory for isolation, identification and serogrouping by standard methods.

### RESULTS AND DISCUSSION

Table 1 summarizes the carrier rates and distribution of serogroups for FY 1979. The overall carrier rate of 32.5% is very close to the rate of FY 1978 (34.4%). There was little change in the frequency of serogroups isolated. Group B strains were again the most commonly isolated (61.3%). W-135's increased from 7% in FY 1978 to 12% in FY 1979 with Y's falling from 16.2% to 10.9% and 29E's also dropping (from 9% to 6.4%). No other serogroup made up as much as 1% of the strains isolated. Figure 1 shows the month by month frequency of the four most common serogroups.

Carrier rates were once again higher in the winter months than in the summer during FY 1979, with the peak month being March (49% carriers) and the low month June (9% carriers).

There was one disease case at Fort Ord during FY 1979, occurring in April. The organism isolated from the blood culture was Group B. Prophylactic treatment with minocycline was undertaken and no further cases of meningitis were reported.

TABLE I

## MENINGOCOCCAL CARRIER SURVEILLANCE FY 1979

	<u>OCT 78</u>	<u>NOV</u>	<u>DEC</u>	<u>JAN 79</u>	<u>FEB</u>	<u>MAR</u>	<u>APR</u>	<u>7TH MAY</u>	<u>21ST MAY</u>	<u>JUN</u>	<u>JUL</u>	<u>AUG</u>	<u>TOTAL</u>	<u>% OF ISOLATES</u>
NUMBER OF SPECIMENS	100	100	100	100	100	100	100	263	63	78	100	100	1104	
NUMBER POSITIVE	40	38	41	27	35	49	19	11	15	7	41	36	359	
% POSITIVE	40%	38%	41%	27%	35%	49%	19%	17.5%	23.8%	9.0%	41%	36%	32.5%	
SEROGROUP:														
B	18	27	26	16	26	26	12	7	8	4	27	23	220	61.3%
C	1	0	0	0	0	1	0	0	0	0	0	0	2	0.6%
29E	5	2	1	3	2	2	0	0	0	1	4	3	23	6.4%
Y	7	3	7	2	1	4	3	2	3	1	4	1	39	10.9%
X	0	1	0	0	0	0	0	0	0	0	0	0	1	0.3%
M-135	8	1	1	3	4	5	4	2	4	1	5	5	43	12.0%
*NT	1	4	6	2	2	11	0	0	0	0	1	4	31	8.6%

\* The non-typeable category includes those strains not agglutinating with any of the antisera as well as those agglutinating with multiple antisera and those agglutinating in saline.

a One individual from this group had meningococcal disease on 4 April 1979. Group B *Neisseria meningitidis* was recovered from a blood culture from that individual. About 60% of this group was treated with minocycline 1 month prior to this carrier survey.

b This is the same group as was surveyed in early May; the sample date is two weeks later.

c No survey was performed in September.

Figure 1 ~ % Meningococcal Isolates by Serogroup

